Investigating the Effect of ATP on Amyloid Aggregation

E. Kamski-Hennekam, R. Ahmed, J. Huang, G. Melacini*
McMaster University, Hamilton, Canada

E-mail: kamskier@mcmaster.ca

Alpha-synuclein (αS) aggregation is the hallmark of a group of neurodegenerative diseases which include Parkinson's Disease (PD) [1]. The greatest risk factor for PD is aging, but a clear link between an age-related change and αS aggregation remains elusive [2]. Recent studies have suggested that Adenosine Triphosphate (ATP), whose levels decline dramatically with age, can act as a hydrotrope at high – but still biologically-relevant – concentrations to prevent the accumulation of and even dissolve protein aggregates [3]. However, ATP can also enhance the aggregation of amyloid proteins like Tau, therefore its hydrotropic effect is highly protein-specific [4]. Here we use a combination of Nuclear Magnetic Resonance (NMR) spectroscopy and Thioflavin T (ThT) fluorescence studies to characterize the interaction of ATP with αS. We explore how ATP-induced remodeling of the αS monomer conformational ensemble influences early-stage αS aggregation. Finally, we use ThT to investigate how ATP affects late-stage αS aggregation and discuss the possible implications for PD development. Overall, our results indicate that physiologically-relevant ATP concentrations elicit different effects on early- and late-stage αS aggregation and that these effects are modulated by Mg^{2+}, whose levels are often decreased in PD patients [5].

References

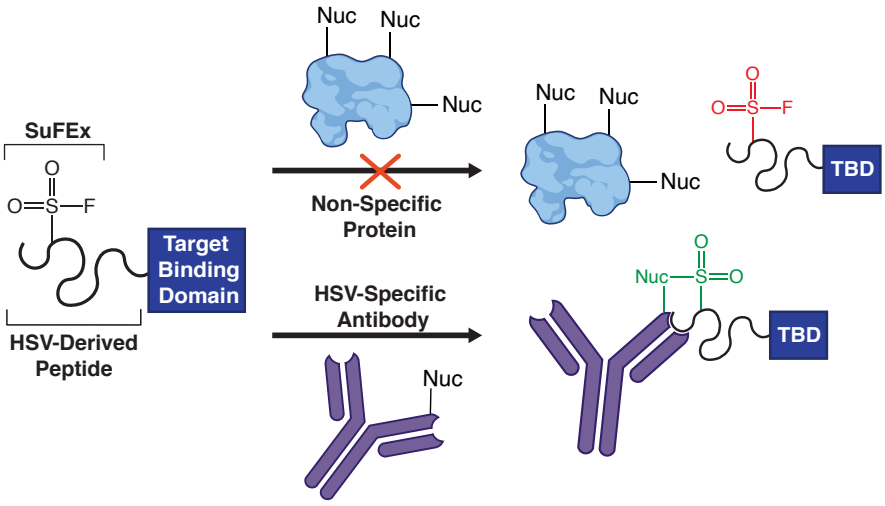
1. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. (1997) Alpha-synuclein in Lewy bodies. *Nature. 388,* 839–40.
2. Reeve A, Simcox E, Turnbull D. (2014) Ageing and Parkinson's disease: Why is advancing age the biggest risk factor? *Ageing. Res. Rev. 14,* 19–30.
3. Patel A, Malinovska L, Saha S, Wang J, Alberti S, Krishnan Y, Hyman AA. (2017) ATP as a biological hydrotrope. *Science. 356,* 753-756.
4. Heo CE, Han JY, Lim S, Lee S, Im D, Lee MJ, Kim YK, Kim HI. (2020) ATP kinetically modulates pathogenic tau fibrillizations. *ACS. Chem. Neurosci. 11,* 3144-3152.
5. Golts N, Snyder H, Frasier M, Theisler C, Choi P, Wolozin B. (2002) Magnesium Inhibits Spontaneous and Iron-induced Aggregation of α-Synuclein. *J. Biol. Chem. 277,* 16116–16123.

A Covalent Immune Proximity-Induction Strategy Using SuFEx Engineered Viral Peptides

H.M. McCann[1], B. Lake[2], M.E. Davola,[3] K. Mossman,[4] A.F. Rullo[5].
[1,2,5]*McMaster University, McMaster Immunology Research Centre, Department of Chemistry and Chemical Biology, Department of Medicine, Hamilton, Ontario*
[3,4]*McMaster University, McMaster Immunology Research Centre, Department of Pathology and Molecular Medicine, Hamilton, Ontario*

E-mail: Mccanh2@mcmaster.ca

Proximity inducing bifunctional molecules modulate the recognition and elimination of cancer cells by the immune system.[1] For this interaction to take place, the ternary complex formed by immunological receptors (e.g., serum antibodies, Fc receptors) with proteins on the cancer cell surface must be sufficiently stabilized through high affinity binding interactions. Relatively unexplored in cancer immunotherapy are viral peptide epitopes, which are known to induce a significant antibody response in a large proportion of our population.[2] By strategically incorporating these peptide epitopes into a proximity-inducing bifunctional format, these endogenous anti-viral antibodies may be directed to cancer epitopes, facilitating a cancer killing response by effector immune cells.[1] Here we demonstrate a covalent proximity induction strategy using a herpes simplex virus (HSV)-derived peptide, incorporated into a covalent bi-functional molecule. Using sulfur (VI) fluoride exchange (SuFEx) chemistry, we explored how ligand directed "effective molarity-EM" allowed for specific covalent labelling of antibodies to achieve an "infinite affinity" binding interaction. We further demonstrate that this covalent proximity-induction strategy efficiently stabilized ternary complexes between effector and target cells with low nM potency. With the ability to covalently recruit endogenous anti-HSV from human IgG, this covalent peptide strategy has potential for therapeutic use *in vivo*, and broad applicability for other viral peptide epitopes.



References

1. Lake, B.; Serniuck, N.; Kapcan, E.; Wang, A.; Rullo, A. F., Covalent Immune Recruiters: Tools to Gain Chemical Control Over Immune Recognition. *ACS Chemical Biology* **2020,** *15* (4), 1089-1095.
2. James, C.; Harfouche, M.; Welton, N. J.; Turner, K. M.; Abu-Raddad, L. J.; Gottlieb, S. L.; Looker, K. J., Herpes simplex virus: global infection prevalence and incidence estimates, 2016. *Bulletin of the World Health Organization* **2020,** *98* (5), 315-329.

Pd-Ir and Aptamer-based Immunoassay for SARS-CoV-2

R Liu,[1] Y Li*[1,2]
[1] *Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Canada*
[2] *Department of Biochemistry and Biomedical Sicences, McMaster University, Hamilton, Canada*
E-mail: liur108@mcmaster.ca

Currently the world is being challenged by a public health emergency caused by the coronavirus pandemic (COVID-19). Rapid, accurate, and high-throughput testing is vital to transmission control, clinical diagnosis, and therapy of viral infections. The enzyme-linked immunosorbent assay (ELISA) is a microwell-plate-based assay technique for colorimetric or quantitative detection of the virus via detecting either virus antibodies or antigens.[1] In a traditional ELISA, horseradish peroxidase (HRP) is a commonly used enzyme that is conjugated to antibodies and specifically generates color signals by catalyzing colorimetric substrates. However, wide practical applications of protein enzymes remain a challenge mainly due to susceptibility to denaturation and digestion, the complexity of preparation and purification, and loss of catalytic activity during storage.[2] Pd-Ir core-shell nanocubes, one of the peroxidase-mimicking nanozymes, exhibit promising features including high catalytic efficiency, ease of production, stability, and uniformity of synthesis. These advantages over protein enzymes make Pd-Ir nanocubes attractive for immunoassays. Aptamers have been proposed as a potential substitute for conventional antibodies due to their similar recognition properties in bioanalysis applications, especially immunoassays.[3] Herein, we proposed Pd-Ir nanocubes and aptamer-based immunoassay for SASR-CoV-2 detection. In this ELISA-like sandwich format assay, aptamers were utilized as the recognition element due to its high sensitivity and specificity for SASR-CoV-2. In this project, our goal is to establish an easy to perform, stable, reliable, and sensitive biosensing method for COVID-19, which will become a good alternative even superior to antibody-based immunoassays due to extraordinary advantages of Pd-Ir and aptamers over protein enzymes and antibodies, respectively.

References

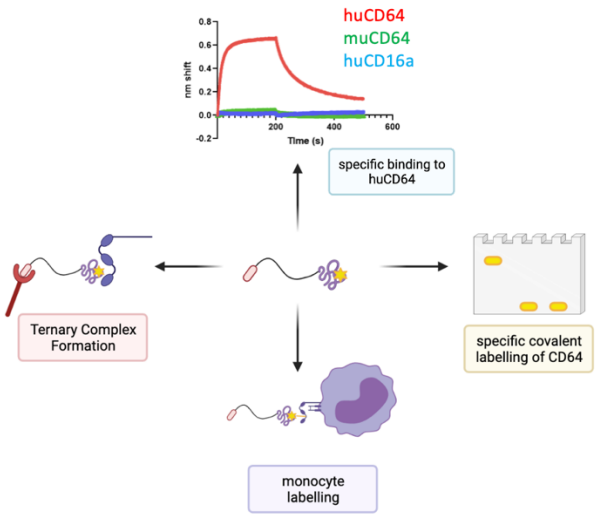
(1) Carter, L. J.; Garner, L. V; Smoot, J. W.; Li, Y.; Zhou, Q.; Saveson, C. J.; Sasso, J. M.; Gregg, A. C.; Soares, D. J.; Beskid, T. R.; et al. Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS Cent. Sci.* **2020**, *6* (5), 591–605.
(2) Xia, X.; Zhang, J.; Lu, N.; Kim, M. J.; Ghale, K.; Xu, Y.; McKenzie, E.; Liu, J.; Ye, H. Pd–Ir Core–Shell Nanocubes: A Type of Highly Efficient and Versatile Peroxidase Mimic. *ACS Nano* **2015**, *9* (10), 9994–10004.
(3) Ruiz Ciancio, D.; Vargas, M. R.; Thiel, W. H.; Bruno, M. A.; Giangrande, P. H.; Mestre, M. B. Aptamers as Diagnostic Tools in Cancer. *Pharmaceuticals* **2018**, *11* (3).

Monocyte Covalent Immune Recruiters; Chemical Tools to Simplify and Enforce Synthetic Immune Recognition

Rebecca C. Turner[1, 2, 3], Anthony Rullo[1,2, 3]
[1]*Department of Chemistry and Chemical Biology, McMaster University*
[2]*McMaster Immunology Research Centre, McMaster University*
[3]*Department of Medicine, McMaster University*
...
E-mail: turner3@mcmaster.ca

Immune recruiters are small molecule based immunotherapeutics designed to redirect endogenous components of the immune system to target cells to elicit an anti-cancer response. Current immune recruiters are hetero-bispecific molecules that simultaneously bind cancer antigens and endogenous antibodies [1]. Complexes formed between the cancer cell, the immune recruiter, and antibodies lead to the recruitment of effector immune cells which carryout anti-cancer responses. The intensity of the anti-cancer response of antibody-based immune recruiters are proposed to be dependent on the number and stability of immune complexes formed between the target and immune cells. Implicit factors such as clearance of the immune recruiter, affinity between the antibody and its target receptor, and endogenous antibody concentrations limit the maximum amount of possible target cell killing.

This research proposes the design and use of monocyte covalent immune recruiters (mCIRs) as the next step in immune recruiting technology. MCIRs target monocytes, which are innate cells of the immune system and responsible for carrying out potent anti-cancer responses. These mCIRS use a scaffold fitted with a reported cyclic peptide to target CD64, a constitutively expressed receptor involved in monocyte activation [2]. The mCIRs have been equipped with a fluorosulfonate-modified tyrosine to covalently label CD64. This project looks to evaluate differences in immune activation due to direct engagement with monocytes and potential enhancement due to the covalent stabilization of the interaction. Ultimately, the pursuit of mCIRs could provide insight to how simplified synthetic immune recruitment can enhance anti-cancer responses.



References

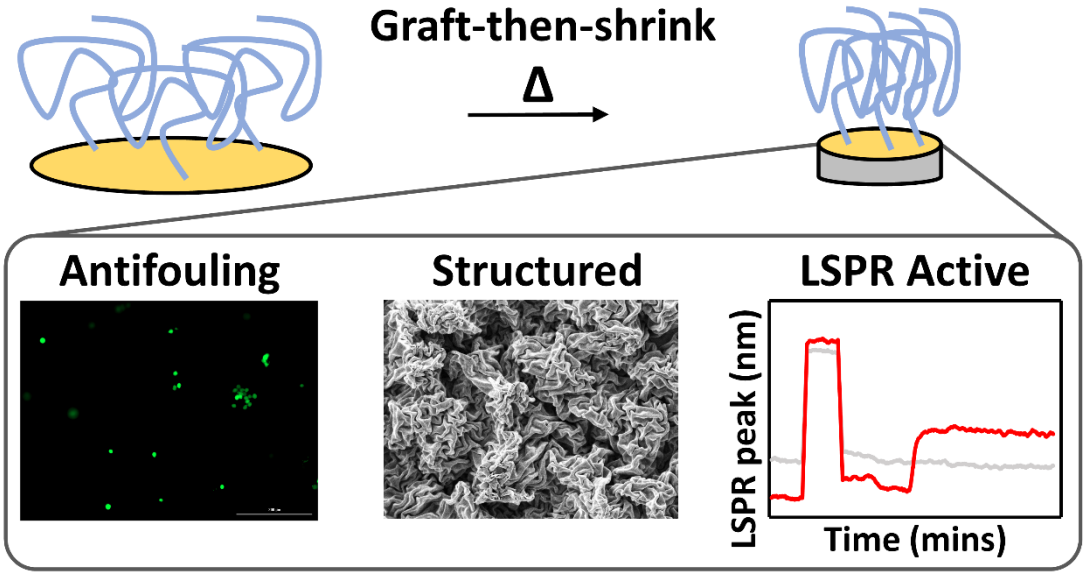
(1) Lake, B.; Serniuck, N.; Kapcan, E.; Wang, A.; Rullo, A. F. Covalent Immune Recruiters: Tools to Gain Chemical Control Over Immune Recognition. *ACS Chem Bio* **2020**. https://doi.org/10.1021/acschembio.0c00112.

(2) Bonetto, S.; Spadola, L.; Buchanan, A. G.; Jermutus, L.; Lund, J. Identification of Cyclic Peptides Able to Mimic the Functional Epitope of IgG1-Fc for Human FcγRI. *FASEB J.* **2009**, *23* (2), 575–585. https://doi.org/10.1096/fj.08-117069.

Title: Simple fabrication of low-fouling LSPR biosensors on shrinkable substrates

A.H. Jesmer[1], R.G. Wylie[1,2]
[1]*Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Canada*
[2]*School of Biomedical Engineering, McMaster University, Hamilton, Canada*
...
E-mail: jesmera@mcmaster.ca

A “Graft-then-Shrink” method was developed for fabricating polymer coated wrinkled Au surfaces that simultaneously improved antifouling properties and generated, first of their kind wrinkled Au localized surface plasmon resonance (LSPR) biosensors. Graft-then-Shrink involves grafting polymers to shrinkable substrates before shrinking. Here, thiol-terminated zwitterionic polymers are grafted onto Au coated thermally shrinkable polystyrene. Compared to “Shrink-then-Graft” controls, where polymers are immobilized after shrinking, Graft-then-Shrink increased polymer amounts 2.5-fold in a defined footprint and improved anti-fouling properties as demonstrated by an 80% reduction in macrophage adhesion. Graft-then-shrink surfaces were used as LSPR biosensors by embedding into a 96-well plate format microfluidic device, and absorbance measurements were made using a typical absorbance plate reader, without specialized instrumentation or optics. The LSPR sensors had sensitivities of ~200-1000 Δλ/ΔRIU, depending on polymer molecular weight, and Au thickness, comparing favorably to commercial LSPR sensors, and were used to detect biotin-avidin and desthiobiotin-avidin interactions.



The role of ammonium ions in prebiotic RNA polymerization

Alix Dujardin[1,2,3], Renee-Claude Bider[1,2], Breanna Crompvoets[1,2], Sebastian Himbert[1,2], Hannah V. Rheinstadter[1,2], Ralph Pudritz[1,2], and Maikel C. Rheinstadter[1,2]

[1]*Department of Physics and Astronomy, McMaster University, Hamilton, Ontario, Canada*

[2]*Origin Institute, McMaster University, Hamilton, Ontario, Canada*

[3]*Department of Chemistry and Chemical Biology, McMaster University, Hamilton, Ontario, Canada*

...

E-mail: dujardia@mcmaster.ca

The "RNA world" proposed by Gilbert in 1986 [1] has aroused the curiosity of many investigators. Researchers have tried to synthesized RNA without enzyme, but by using different catalysts such as clay [2,3], lipids [4,5], or salts [6].

Here, we propose to investigate the formation of RNA by using hydration-dehydration cycles in the presence of different compounds. This study used a novel simulator chamber to mimic hot-cold and hydration-dehydration cycles with unprecedented resolution and duration.

When analyzed by gel electrophoresis and computer analysis, we found that ammonium salts are an excellent catalyst for RNA synthesis, >150~bp. We used molecular dynamic simulations to show that in the presence of ammonium ions, nucleotides formed tight clusters, and hydrogen bonds between ions and nucleotides. However, each ammonium salt gave a different rate of polymerization. That can be related to the molar Gibbs energy of formation ($\Delta_f G$) of the salt. The more $\Delta_f G$ is favorable, thus small, the better for RNA polymerization. This observation can be explained by the size of the crystal formed. With a low $\Delta_f G$, nucleation will start at many different places, and thus smaller crystals will grow. Nucleotides, which are concentrated on the crystals, are then more homogeneously distributed in the sample.

We also found that the RNA length was not related to the number of cycles. Moreover, irregular cycling, with a more prolonged dehydration phase, increases the polymerization rate.

References

1. Gilbert W. Origin of life: The RNA world. nature. 1986 Feb;319(6055):618-.

2. Ferris JP. Mineral catalysis and prebiotic synthesis: montmorillonite-catalyzed formation of RNA. Elements. 2005 Jun 1;1(3):145-9.

3. Ferris JP. Montmorillonite-catalysed formation of RNA oligomers: the possible role of catalysis in the origins of life. Philosophical Transactions of the Royal Society B: Biological Sciences. 2006 Oct 29;361(1474):1777-86.

4. Rajamani S, Vlassov A, Benner S, Coombs A, Olasagasti F, Deamer D. Lipid-assisted synthesis of RNA-like polymers from mononucleotides. Origins of Life and Evolution of Biospheres. 2008 Feb;38(1):57-74.

5. Toppozini L, Dies H, Deamer DW, Rheinstädter MC. Adenosine monophosphate forms ordered arrays in multilamellar lipid matrices: Insights into assembly of nucleic acid for primitive life. PloS one. 2013 May 7;8(5):e62810.

6. Da Silva L, Maurel MC, Deamer D. Salt-promoted synthesis of RNA-like molecules in simulated hydrothermal conditions. Journal of molecular evolution. 2015 Feb 1;80(2):86-97.