CURRICULUM VITAE

Personal Particulars

Name:	KIN Fan, **ON** (Kenneth)
Date of Birth:	15th August 1985
Place of Birth:	Hong Kong
Nationality:	Chinese
Citizenship:	Hong Kong SAR (US Green Card holder)
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Employment

Jan 2020 – Present

Research Specialist,
Howard Hughes Medical Institute
Investigating molecular mechanisms of various biological pathways (human DNA replication, translational control, chromatin biology, kinases, and transcription factors implicated in cancer biology; COVID-related therapeutic targets), with biochemistry, biophysics, molecular biology, cryo-electron microscopy (cryo-EM) and X-ray crystallography techniques, to identify therapeutic targets and explore their therapeutic potentials based on structure/function relationship, under the supervision of Dr. Leemor Joshua-Tor (laboratory site: Cold Spring Harbor Laboratory)

Nov 2016 – Jan 2020

Research Associate,
Howard Hughes Medical Institute
Investigating the molecular mechanisms of motors proteins involved in human DNA replication initiation, with biochemistry, biophysics, molecular biology, cryo-electron microscopy (cryo-EM) and X-ray crystallography techniques, under the supervision of Dr. Leemor Joshua-Tor (laboratory site: Cold Spring Harbor Laboratory)

Nov 2015 – Nov 2016	**Postdoctoral Fellow,** **NYS Cancer Gene Discovery and Cancer Biology Postdoctoral Training Program (CSHL)** Investigating the molecular mechanisms behind inhibition of the human RNA helicase eIF4A1 by synthetic chemotherapeutic agents, using a combination of biochemistry and X-ray crystallography, under the supervision of Dr. Leemor Joshua-Tor
Mar 2014 – Nov 2015	**Research Associate,** **Howard Hughes Medical Institute** Investigating the molecular mechanisms of motors proteins involved in human DNA replication initiation, with biochemistry, biophysics, molecular biology and X-ray crystallography techniques, under the supervision of Dr. Leemor Joshua-Tor (laboratory site: Cold Spring Harbor Laboratory)

Education

Sep 2009 – Nov 2013 **Graduate student, London Research Institute, Cancer Research U. K.**
(Ph. D. degree awarded by the University College London, U. K.)
Ph. D. thesis research project under the supervision of Dr. John Diffley. Research work was conducted in Clare Hall Laboratories, Cancer Research UK London Research Institute
Project: "Biochemical characterization of MCM helicase activation *in vitro*"

Sep 2007 – Aug 2009 **Master of Philosophy in Biochemistry, the Hong Kong University of Science and Technology (HKUST), Hong Kong**
M. Phil. research project under the supervision of Professor Randy Poon
Project: "The role of MAD2L1BP in the silencing of the spindle-assembly checkpoint and the DNA damage checkpoint"

Sep 2004 – Aug 2007 **Bachelor of Science in Biochemistry (First-class Honors), the Hong Kong University of Science and Technology (HKUST), Hong Kong**
Final year research project under the supervision of Professor Randy Poon

Sep 1997 – Aug 2004 **St. Mark's School**, **Hong Kong**

Awards, Academic Distinctions and Fellowships

2015 – 2016	NYS Cancer Gene Discovery and Cancer Biology Postdoctoral Training Program (CSHL), National Institutes of Health (NIH)
2010 – 2012	Boehringer Ingelheim Fonds PhD Fellowship
2008	The George K Lee Foundation Scholarship
2007 – 2008	Best Teaching Assistant Award (Department of Biochemistry)
2006 – 2007	Dean's List (Fall and Spring Semesters)
2006	Chiap Hua Cheng's Foundation Scholarship
2004 – 2005	Dean's List (Fall and Spring Semesters)

Experience

Research Specialist (Howard Hughes Medical Institute)

Projects: Investigating molecular mechanisms of various biological pathways for identification of therapeutic targets and exploration of their therapeutic potentials based on structure/function relationship

-Conceptualize research objectives and devise strategies for the research projects with my supervisor Dr. Leemor Joshua-Tor.

-Independently formed hypotheses, designed, performed and trouble-shot biochemical experiments, and collaborated with scientific coworkers to perform structural biology experiments (X-ray crystallography and cryo-EM) on a hypothesis-driven basis.

-Conduct literature reviews to identify the most suitable research methods to drive research projects forward productively.

-Generated protein expression constructs of bacterial strains (for expressions in *E. coli*) and baculoviruses (for expression in Sf9 and Hi5 insect cells); designed and optimized protein purification protocols; performed trouble-shooting procedures to improve proteins yield and quality.

-Established biochemical/biophysical assays for monitoring the functional activities of the various target proteins (e. g. Fructosamine-3-kinase, FN3K, and Papain-Like Protease, PLpro; Spike proteins in COVID; human ATPases in the DNA replication pathway; transcription factors implicated in cancer therapeutic targeting, etc.) including radioactive ATP hydrolysis assays, radioactive/non-radioactive electrophoretic mobility shift assays (EMSAs), fluorescence-based high-throughput assays, micro-scale thermophoresis (MST) and surface plasmon resonance (SPR).

-Conducted research on understanding the catalytic mechanisms of FN3K, which has been implicated as an oncogenic target in the NRF2 pathway in several cancers. Our structural data shed lights on rational design of compounds targeting FN3K, and provide a platform for robust screening of potential small molecules (manuscript in preparation).

-Performed preliminary studies on the characterizations of potential therapeutic targets related to COVID: (1) established high-throughput PLPro assays and executed screening experiments against a panel of compounds designed based on protein structure from collaborators. (2) purified various forms of spike proteins for a collaborative project on understanding the molecular interplay between spike proteins and neutrophils, which has implications in prognosis of disease severity (manuscript in preparation).

-Conducted follow-up studies on human ORC project and further characterize the involvement of ATP-binding and ATP-hydrolysis in the functionality of ORC with biochemistry and cryo-EM. This work provided further insights into the molecular basis underlying the genetic mutations observed in Meier-Gorlin Syndrome (MGS) (manuscript in preparation).

-Established purification protocols for human replication proteins (e. g. CDC6, CDT1, MCMs, etc.). Further work will focus on biochemical characterization and structural investigations of these proteins with cryo-EM.

-Other projects that are on-going but cannot be disclosed due to confidentiality.

-Manuscript preparation and submission of various scientific work for publications in peer-reviewed journals.

-Supervision and tutoring of junior laboratory coworkers (e. g. lab technicians; undergraduate students who performed short-term projects in the laboratory).

Research Associate (Howard Hughes Medical Institute)
Project: Investigating the molecular mechanisms of motors proteins involved in human DNA replication initiation
-Established research strategies for the research project with my supervisor Dr. Leemor Joshua-Tor.
-Independently formed hypotheses, designed and performed biochemical and biophysical experiments, and collaborated with scientific coworkers to perform structural biology experiments (X-ray crystallography and cryo-EM) on a hypothesis-driven basis.
-Conduct literature reviews for determining the most suitable research methods to drive research projects forward productively.
-Generated protein expression constructs of bacterial strains (for expression in *E. coli*) and baculoviruses (for expression in Sf9 and Hi5 insect cells); designed and optimized protein purification protocols; performed trouble-shooting procedures to improve proteins yield and quality.
-Established biochemical and biophysical assays for monitoring the functional activities of the human origin recognition complex (ORC) proteins, including radioactive ATP hydrolysis assays; radioactive/non-radioactive electrophoretic mobility shift assays (EMSAs); thin-layer chromatography (TLC), dynamic light scattering (DLS), micro-scale thermophoresis (MST) and surface plasmon resonance (SPR).
-Conducted research on understanding the architecture of the human ORC (the first human ORC protein structure ever solved), and to use these data to design and perform biochemical and biophysical experiments to demonstrate the effects of previously reported genetic mutations of human ORC implicated in genetic disorder. I have contributed to be the first scientific group in the world to provide a mechanistic explanation to the molecular basis of the genetic disorder Meier-Gorlin Syndrome (MGS).
-Published results of this work in a peer-reviewed paper (Tocilj *et al.*, 2017).
-Wrote and published a comprehensive review article about the structural view of the initiators for chromosome replication, with special focuses on human ORC and the replicative clamp-loader RFC complex (On *et al*., 2018).
-This review was published as an invited review, in the protein-nucleic acid interactions section of the journal Current Opinion in Structural Biology, published by the Elsevier Press, in December 2018.

Postdoctoral Fellow (CSHL)
(NYS Cancer Gene Discovery and Cancer Biology
Postdoctoral Training Program, CSHL)
Project: Investigating the molecular mechanisms behind inhibition of the human RNA helicase eIF4A1 by synthetic chemotherapeutic agents
-Formulated scientific plans for the project with my supervisor Dr. Leemor Joshua-Tor and our collaborators Dr. Hans-Guido Wendel and Dr. Derek Tan at the Memorial Sloan Kettering Cancer Center (MSKCC).
-Contributed to the portion of the research proposal regarding structural biology and biochemistry experimental approaches for the Starr Cancer Consortium grant application (approved).
-Independently executed research procedures, perform experiments, collected and interpreted biochemical and X-ray diffraction data, followed-up with experiments for hypotheses testing.
-Generated protein expression constructs of bacterial strains (for expression in *E. coli*) and baculoviruses (for expression in Sf9 and Hi5 insect cells); designed and optimized protein purification protocols; performed trouble-shooting procedures to improve proteins yield and quality.
-Devise biochemical and biophysical assays for monitoring the functional activities of the human RNA helicase eIF4A proteins, including radioactive ATP hydrolysis assays; radioactive/non-radioactive electrophoretic mobility shift assays (EMSAs); thin-layer chromatography (TLC), dynamic light scattering (DLS) and
micro-scale thermophoresis (MST).
-Investigated the effects of the synthetic inhibitor CR-31B on highly purified human eIF4A1 proteins *in vitro*, and demonstrated conditions that were required for such effects to occur (the presence of strong bindings by specific RNA substrates).
-Established stable multi-component complexes of the human eIF4A1 proteins, synthetic inhibitor CR-31B and several RNA substrates, and subjected these samples to protein crystallization trials.

Doctor of Philosophy

Project: Biochemical Characterization of MCM Helicase Activation In Vitro

-Devised scientific strategies for the research project with the guidance from my PhD advisor Dr. John Diffley.

-Independently implemented research plans, conducted experiments, analyzed data, performed trouble-shooting, formed and tested hypotheses analytically.

-Wrote a research proposal for the application of the Boehringer Ingelheim Fonds PhD Fellowship award program and successfully secured the award (<10% success rate).

-Generated protein expression constructs, yeast strains (*S. cerevisiae*), bacterial strains (*E. coli*) and baculoviruses (for expression in Sf9 insect cells), and established protein purification and characterization protocols (including yeast protein purification from 10 L and 100 L fermenters).

-Established and characterized a novel *in vitro* biochemical system recapitulating DNA replication *in vivo* (On *et al.*, 2014).

-Examined the role of phosphorylations on the replicative helicase MCM in DNA replication initiation with such biochemical system and electron microscopy.

-Perform literature reviews to identify experimental procedures applicable to my research.

-Presented my research as a talk at the Keystone Symposium at
"DNA Replication and Recombination" conference, Mar 3 – Mar 8, 2013,
Banff, Alberta Canada.

-Published results of research work in a peer-reviewed paper (On *et al*., 2014).

-Wrote and published a comprehensive review article about the regulation of eukaryotic DNA replication (Siddiqui *et al*., 2013). This article was published as a book chapter in a textbook published by the Cold Spring Harbor Press in 2013.

Master of Philosophy

Project: The Role of MAD2L1BP in the Silencing of the Spindle-assembly Checkpoint and the DNA Damage Checkpoint

-Discussed research strategies for the project with my MPhil advisor Prof. Randy Poon.

-Established protocols for studying mammalian cell cycle checkpoints interplay *in vivo* (e. g. live-cell imaging, immunofluorescence microscopy, flow-cytometry assays, cell viability-monitoring assays, colony formation assays, etc.).

-Performed molecular cloning of various expression constructs of target proteins after mutagenesis for expressions and characterizations in mammalian cells.

-Generated mammalian stable cell-lines for cell cycle analysis experiments.

-Examined the role of spindle-assembly checkpoint in safeguarding genomic integrity in mammalian cells after DNA damage checkpoint bypass.

-Performed molecular biology techniques including molecular cloning and mutagenesis, Western blotting, live-cell imaging, immunofluorescence studies, operation of flow cytometer and protein expression and purification from bacteria (*E. coli*).

-Reviewed literature and accommodated methodology suitable for my research project.

-Published results of research work in peer-reviewed papers
(Ma *et al.*, 2007; Chan *et al.*, 2008a; Chan *et al.*, 2008b; On *et al.*, 2011; Ma *et al.*, 2012).

Skills

Scientific Techniques:

-Molecular cloning, protein engineering, site-directed mutagenesis and truncations generation with traditional cloning with restriction enzyme digestion-ligation, and Sequence and Ligation Independent Cloning (SLIC);
-Establishment and optimization of protein expression and purification protocols, using expression systems including *Saccharomyces cerevisiae*, *Escherichia coli*, baculovirus-infection of insect cells (Sf9 and Hi5), and transfections and target protein expression human cells (HEK);
-Scientific capabilities to the designing of novel biochemical experiments based on the biological questions in a hypotheses-driven manner and the delicacy of conducting biochemical experiments;
-Various column chromatographic manipulations and AKTA systems operation (AKTA Pure, AKTA Explorer, AKTA Prime);
-Biochemical and biophysical techniques for assessment of protein quality and activity characterization, including high-throughput, plate reader-based biochemical experiments, thin-layer chromatography (TLC), dynamic light scattering (DLS), micro-scale thermophoresis (MST) and surface plasmon resonance (SPR); experienced in working with radioactivity;
-Protein analysis with SDS-PAGE/native PAGE;
-Cell biological techniques including immunofluorescence microscopy, live-cell imaging and basic operation of flow cytometer.
Molecular biology techniques.

Computer Literacy:

Adobe: Illustrator and Photoshop.
Microsoft Office: Excel, PowerPoint, Word, Outlook.
Apple iWork: Keynote, Numbers, Pages.
Scientific data analyzer: GraphPad Prism.
Molecular visualization and model building tools: PyMOL, ChimeraX, Coot.
X-ray crystallographic data processing software (basic operation):
X-ray Detector Software (**XDS**).
Cryo-electron microscopy data processing software (basic operation):
Warp, cryoSPARC.

Languages:

English (Professional working proficiency)
Cantonese (Native)
Mandarin (Limited working proficiency)

Publications

(Other manuscripts with collaborators, either submitted or in preparation, are not shown due to confidentiality consideration)

***On, K. F.**, * Garg, A. and Joshua-Tor, L.
**contributed equally*
Structural and mechanistic insights into the human transcription factor NRF2.
Manuscript in preparation (2023)

***On, K. F.**, *Garg, A. and Joshua-Tor, L.
**contributed equally*
Molecular mechanisms and therapeutic potentials of fructosamine-3-kinase (FN3K) catalysis.
Manuscript in preparation (2023)

***On, K. F.**, *Jaremko, M., Thomas, D., Stillman, B. and Joshua-Tor, L.
**contributed equally*
The dynamic domains of the human origin recognition complex (ORC).
Manuscript in preparation (2022)

On, K. F., Jaremko, M., Stillman, B. and Joshua-Tor, L.
A Structural View of the Initiators for Chromosome Replication.
Current Opinion in Structural Biology 53: 131-139 **(2018)**

Tocilj, A., **On, K.F.**, Yuan, Z., Sun, J., Elkayam, E., Li, H., Stillman, B., Joshua-Tor, L.
Structure of the active form of human origin recognition complex and its ATPase motor module.
eLife 2017;6:e20818 **(2017)**

On, K.F., Beuron, F., Frith, D., Snijders, A.P., Morris, E.P., and Diffley, J.F.X.
Prereplicative complexes assembled *in vitro* support origin-dependent and independent DNA replication.
EMBO Journal 33(6): 605-20 **(2014)**

Siddiqui, K., **On, K.F.**, and Diffley, J.F.X.
Regulation of eukaryotic DNA replication. (book chapter)
Cold Spring Harbor Perspectives in Biology 2013;5:a012930 **(2013)**

Ma, H.T., Chan, Y.Y., Chen, X., **On, K.F.**, and Poon, R.Y.C.
Depletion of p31comet protein promotes sensitivity to antimitotic drugs.
Journal of Biological Chemistry 287(25): 21561-9 **(2012)**

On, K.F., Chen, Y., Ma, H.T., Chow, J.P.H., and Poon, R.Y.C.
Determinants of mitotic catastrophes upon abrogation of the G2 DNA damage checkpoint by UCN-01.
Molecular Cancer Therapeutics 10(5): 784-94 **(2011)**

Chan, Y.W., **On, K.F.**, Chan, W.M., Wong, W., Siu, H.O., Hau, P.M., and Poon, R.Y.C.

The kinetics of p53 activation versus cyclin E accumulation underlies the relationship between the spindle-assembly checkpoint and the postmitotic checkpoint.
Journal of Biological Chemistry 283(23): 15716-23 **(2008)**

Chan, Y.W., Ma, H.T., Wong, W., Ho, C.C., **On, K.F.**, and Poon, R.Y.C.
CDK1 inhibitors antagonize the immediate apoptosis triggered by spindle disruption but promote apoptosis following the subsequent rereplication and abnormal mitosis.
Cell Cycle 7(10): 1149-61 **(2008)**

Ma, H.T., **On, K.F.**, Tsang, Y.H., and Poon, R.Y.C.
An inducible system for expression and validation of the specificity of short hairpin RNA in mammalian cells.
Nucleic Acids Research 35(4): e22 **(2007)**