Alex Siegel

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EDUCATION & TRAINING

Postdoctoral Scholar in Chemistry, California Institute of Technology (2017-present)

Ph.D. Biophysics and specialization in Chemical Biology, University of California, Berkeley (2016)

B.S. Biology and Chemistry double major, graduated with honors, California Institute of Technology (2008)

RESEARCH EXPERIENCE

Postdoctoral research with Prof. Shu-ou Shan at Caltech **2017 – present**

In my postdoctoral work, I studied how two molecular chaperones assist with the folding of their client proteins: cpSRP43, a small, ATP-independent chaperone important for the delivery of light harvesting complex proteins (LHCPs) critical for photosynthesis and human ClpB (Skd3), a mitochondrial chaperone and disaggregase.

● I used crosslinking, mass spectrometry, and mutagenesis studies to map the contact surface between cpSRP43 and the transmembrane domains of its LHCP clients (McAvoy et al, 2018).

● I identified a novel class of cpSRP43 substrates involved in chlorophyll synthesis (Wang et al, 2018) and found that during heat stress cpSRP43 changes its client selectivity towards these enzymes exactly when this additional protection is needed (Ji et al, 2021).

● I used NMR, EPR, and H/D exchange mass spectrometry to show how allosteric regulation of cpSRP43 drives a disorder-to-order transition to direct it towards LHCP clients (Siegel et al, 2020) or to protect chlorophyll synthesizing clients from heat stress (Siegel et al, 2024 in prep; Provisional Patent ID: CIT-9049-P).

● I solved the cryo-EM structure of the human ClpB homolog, Skd3, and showed that it forms two complex forms with different activities: a hexameric ATPase machine that applies force to disaggregate clients, and a dodecameric cage that provides a protected environment for refolding (Gupta et al, 2023).

My work on these chaperones reveals different ways that chaperones select and bind their clients and how their chaperone cycle can be regulated to capture and release clients at the proper time and place in the cell.

Doctoral research with Prof. David Wemmer at UC Berkeley **2009 – 2016**

In my doctoral work, I studied the mechanism of bacterial RNA polymerase (RNAP) transcription initiation. One of the modular σ factor subunits of RNAP, σ^{54}, regulates the transcriptional response to stress but unlike other σ factors it requires an additional AAA+ ATPase transcriptional activator, NtrC, to be activated.

● I used NMR and other biophysical methods, to identify the minimal 'activator interacting domain' (AID) on σ^{54}, an amphipathic helix that forms the first encounter with the NtrC transcriptional activator exclusively in the ATP-bound state (Siegel et al, 2016).

● I used molecular tweezers and biochemistry to support a mechanistic model where ATP hydrolysis in NtrC threads the AID to apply the force that ultimately melts DNA and exposes the single stranded templates for transcription (Siegel, 2016, dissertation).

● I characterized a piezophillic NtrC homolog by high pressure NMR to identify pressure-dependent conformational changes that activate it (Siegel, 2016, dissertation).

Undergraduate Research at Caltech **2004 – 2008**

As an undergraduate, I worked on diverse projects in cognitive neuroscience, biochemistry, and bioengineering.

● I collected multiphoton microscopy images on developing mouse embryos to contribute to a mouse developmental atlas. With *Prof. Scott Fraser,* Caltech, 2004.

● I designed, animated, and tested a series of shape-based movies that help to identify autism spectrum disorder in children independent of facial expressions cues. With *Prof. Ralph Adolphs,* Caltech, 2005.

● I screened a library of inhibitors to Metacaspase 1 in *Plasmodium falciparum* as targets for anti-malarial drugs. With *Prof. James McKerrow,* UCSF, 2006.

● I observed the neuronal cell response to terahertz radiation as a potential therapeutic method for the selective killing of cancer cells. With *Prof. Jan Stake*, Caltech, 2007.

PUBLICATIONS

***** Indicates equal contribution first author publications.*

Siegel, A, Shan, SO Switchable client specificity in a dual functional chaperone coordinates light harvesting complex biogenesis. *In preparation for submission. (2024)*

Siegel, A*, Singh, J*, Qin, PZ & Shan, SO EPR Studies of Chaperone Interactions and Dynamics. in Biophysics of Molecular Chaperones (eds. Hiller, S., Liu, M. & He, L.) 242–277 (Royal Society of Chemistry, 2023). doi:10.1039/BK9781839165986-00242.

Gupta, A*, Lentzsch AM*, **Siegel A***, Yu Z, Chio US, Cheng Y, & Shan SO. Dodecamer assembly of a metazoan AAA+ chaperone couples substrate extraction to refolding. *Science Advances*. 9(19), eadf5336 (2023).

Ji S*, **Siegel A***, Shan, S., Grimm, B. & Wang, P. Chloroplast SRP43 autonomously protects chlorophyll biosynthesis proteins against heat shock. *Nature Plants*. 7, 1420-1432 (2021).

Siegel A*, McAvoy C*, Lam V, Liang FC, Kroon G, Miaou E, Griffin P, Wright P, Shan SO. A Disorder-to-Order Transition Activates an ATP-Independent Membrane Protein Chaperone. *Journal of Molecular Biology*. 432, 166708 (2020).

McAvoy C, **Siegel A**, Piszkiewicz S, Miaou E, Yu M, Nguyen T, Moradian A, Sweredoski MJ, Hess S, Shan SO. Two Distinct Sites of client protein interaction with the chaperone cpSRP43. *Journal of Biological Chemistry*. RA118, (2018).

Wang P, Liang FC, Wittmann D, **Siegel A**, Shan SO, Grimm B. Chloroplast SRP43 acts as a chaperone for glutamyl-tRNA reductase, the rate-limiting enzyme in tetrapyrrole biosynthesis. *Proceedings of the National Academy of Sciences*. 115(15), E3588-96 (2018).

Siegel AR, Wemmer DE. Role of the σ^{54} Activator Interacting Domain in Bacterial Transcription Initiation. *Journal of Molecular Biology*. 428(23), 4669-85 (2016).

Siegel AR. Mechanisms of σ^{54} bacterial transcription activation. *Doctoral Dissertation University of California, Berkeley.* Publication No. 10192026. ProQuest Dissertations Publishing (2016).

PATENTS

Patent ID: CIT-9049-P *Provisional patent application* related to neurodegenerative diseases (Details upon request).

PRESENTATIONS

**Oral presentation, †Poster presentation ‡Session chair*

33rd Western Photosynthesis Conference*†‡, "Regulated capture and delivery of the light harvesting complex proteins by chloroplast SRP." Biosphere 2, AZ (2024).

ASBMB annual meeting in Experimental Biology*†‡, Spotlight Session, "Client Specificity of an ATP-independent Chaperone is Regulated by a Temperature Sensitive Switch", Siegel AR, Ji S, McAvoy CZ., Lam V, Liang FC, Kroon G, Miaou E, Griffin P, Grimm B, Wang P, Wright P, Shan SO, Philadelphia, PA (2022).

ASBMB Science in a Flash*, Flash Talk competition, "A molecular thermostat that protects plants from heat." Siegel AR., Shan SO., Virtual Meeting (2022)

Center for Molecular and Cellular Medicine*, Biochemistry Seminar Series, "A molecular thermostat switches on alternative chaperone activity during heat stress.", Siegel AR, Ji S, Grimm B, Wang P, Shan SO, Caltech (2021)

EMBO workshop*, New challenges in protein translocation across membranes, "A disorder-to-order transition activates the chloroplast signal recognition particle", Siegel AR, McAvoy CZ, Liang FC, Kroon G, Wright P, Shan SO, Virtual Meeting (2021).

Center for Molecular and Cellular Medicine*, Biochemistry Seminar Series, "Regulated substrate capture and release by a membrane protein chaperone", Siegel AR, McAvoy CZ, Liang FC, Kroon G, Wright P, Shan SO, Caltech (2020).

Gordon Research Conference in Computational Aspects of Biomolecular NMR†**, "Role of the σ^{54} Activator-Interacting Domain in Bacterial Transcription Initiation", Siegel AR, Wemmer DE., Lucca, Italy (2015).

Structure Supergroup*, Biophysics Seminar Series, "Mechanisms of σ^{54} Bacterial Transcription Initiation", Siegel AR, Wemmer DE, UC Berkeley (2014).

Biophysics Annual Retreat†, "Mechanisms of σ54 Bacterial Transcription Initiation" Siegel AR, Wemmer DE, UC Berkeley (2014).

Structure Supergroup*, Biophysics Seminar Series, "Mechanisms of Bacterial Transcription Activation", Siegel AR, Wemmer DE, UC Berkeley (2012).

Biophysics Annual Retreat†, "The σ^{54} Switch at the Activator Binding Domain." Siegel AR, Wemmer DE, UC Berkeley (2010).

4th IEEE EMBS Int. Sym. On Medical Devices and Sensors, "Investigations Towards Thermal and Non-Thermal Effects of THz Radiation on Biological Systems," Siegel P, Pikov V, Stake J, Siegel A. Cambridge, UK, (2007).

Institute of Cell Biophysics, "Millimeter wave exposure of cell cultures to determine thresholds for apoptosis," Siegel P, Pikov V, Stake J, Siegel A. Pushchino, Russia (2007).

Summer Undergraduate Research Fellowship Seminar*, "Determining the Specificity of Metacaspase 1 in Plasmodium berghei." Siegel AR, Sajid M, McKerrow J, UCSF (2006).

Cognitive Neuroscience Society Meeting†, "Perceiving Emotions from the Interactions of Moving Shapes", Siegel AR., Castelli F., Adolphs R. San Francisco, CA (2006).

Summer Undergraduate Research Fellowship Seminar*, "Perceiving Emotions from the Interactions of Moving Shapes." Siegel AR., Castelli F., Adolphs R. Caltech (2005).

Institutes for the 21st Century, High School Internship†, "Techniques in Biological Imaging," Siegel A., Waters C., Fraser S, Caltech (2004).

HONORS & AWARDS

Western Photosynthesis Conference Travel Award to 33rd WPC (2024)

Caltech Certificate of Teaching Interest for completion of yearlong practical teaching course (2023)

ASBMB Graduate Student/Postdoc Travel Award to ASBMB 2022 Annual Meeting (2022)

Kirschstein-NRSA trainee fellowship in Molecular Biophysics, at UC Berkeley (2009-2011)

Biology Undergraduate Teaching Award at Caltech (2008)

ASCIT Honor Key at Caltech (2008)

TEACHING

Courses taught, University**:** **Lead Course Instructor, †Discussion Section Instructor, ‡Lab Course Instructor*

Advanced Molecular Biology Lab‡, UC Berkeley (2016). Students learned and carried out introductory molecular biology techniques (e.g. cloning, protein expression) and then designed their own experiments to study the yeast kinesin Cin8.

Biophysics module on protein NMR spectroscopy*, UC Berkeley (2012). I designed and taught a 1 unit, 5-week short course on protein NMR to interested early graduate students.

Introductory Chemistry Lab‡, UC Berkeley (2011). I led both lab classes and discussion sessions for two classes of ~30 students, along with office hours.

Biophysical Chemistry†, UC Berkeley (2010). TAed two sections of CHEM110 of ~30 students each. Prepared weekly section presentations covering introductory topics in molecular biology. Held weekly office hours. Wrote some of the homework and exam problems for the course.

Introductory Biology†, Caltech (2007 & 2008). TAed a section of ~20 undergraduates each year. Prepared weekly section presentations covering introductory topics in molecular biology. Held weekly office hours. Wrote some of the homework and exam problems for the course.

MENTORING

Research supervision as primary mentor: 7 graduate, 6 undergraduate, 6 high school (names abbreviated for privacy reasons)

Graduate: **Graduate student rotation primary mentor*

B.Y. * – Conformational changes in tailor made mutant chaperones (3 mo. 2023)

W.S.* – Characterization of cpSRP43's dual conformations (3 mo. 2022)

Y.L* – Split luciferase system for the detection of nanomolar binding affinity (3 mo. 2021)

J.P-G.* – Client binding of a superactive chaperone from directed evolution (3 mo. 2021)
J.P.* – Force-dependence of the σ54 core binding domain using molecular tweezers (1 yr. 2013-2014)
C.C.* – Intrinsic disorder of σ^{54} domains in the RNA polymerase holoenzyme by NMR (3 mo. 2014)
J.B.* – Dynamics of M. tuberculosis lysine acetyltransferase (Rv0998) by NMR (3 mo. 2011)

Undergraduate: **Undergraduate thesis primary mentor, †Summer research fellowship (SURF) primary mentor*
A.H.† – Measuring chaperone-client binding by fluorescence lifetime spectroscopy (3 mo. 2024)
S.C.† – Conformational states of cpSRP43 by fluorine NMR. (6 mo. 2019)
M.Y.† – Client interactions with cpSRP43 leading to co-publication. (3 mo. 2017)
D.C-M.* – Activation of σ^{54} by AAA+ ATPase enhancer binding proteins. (1 yr. 2012-2013)
K.O.* – Mechanism NtrC4 receiver domain activation by NMR. (2yr. 2011-2013)
K.A-J.* – Force-dependent unfolding of the σ^{54} by molecular tweezers. (3 yr. 2010-2013)

High school: **Science Fair primary mentor, †High school thesis primary mentor*
E.H.*,* – Identify the client binding state of a novel cpSRP43 client. (2 yr. 2021-2023)
A.C.† – Heat dependence of cpSRP43 client binding. (2 yr. 2021-2022)
J.L* – Conformational states of cpSRP43 by fluorine NMR. (2 yr. 2019-2021)
A.P.* – Conformational states of cpSRP43 by fluorine NMR. (2 yr. 2019-2021)
A.D. – Mutational assignment of cpSRP43 NMR spectrum (2 yr. 2017-2019)
F.C.† – Mutational assignment of cpSRP43 NMR spectrum (2 yr. 2017-2019)

ACADEMIC SERVICE

CCE Postdoc Committee – Representative on the committee liaising between postdoctoral scholars with the Chemistry and Chemical Engineering Division. (2022-2023)

Caltech Postdoctoral Association (CPA) – Secretary/Vice chair of the CPA. Coordinated activities to improve the postdoctoral experience at Caltech, including: increased funding for the CPA events, hundreds of planned career and social events, advocacy for postdoc salaries and benefits, and better onboarding of new postdocs to the administration, coordination and information exchange of postdocs across all 6 academic divisions, etc. (2018-2022)

ExploreCaltech – Part of the team organizing the ExploreCaltech (formerly Science for March) events that brought together Caltech scientists to interactive booths and scientific talks geared for the general public especially local elementary school children. (2019, 2020)

ISEF Judge – Served as a judge in biochemistry and biophysics for the Regeneron International Science and Engineering Fair. Responsible for interviewing students about their projects and discussing awards with the judge committee. (2017)

UC Berkeley Graduate Assembly Delegate (GA) – Served as the representative from the Biophysics Graduate Group to the GSA. The assembly gathered feedback from departments and met monthly to vote on graduate student action for issues facing UC Berkeley (2011-2012)

Caltech Academic Research Council (ARC) – Representative and Secretary on the ARC responsible for gathering feedback and reporting to faculty. The committee then made recommendations to improve undergraduate core curriculum at Caltech. (2005-2007)

PROFESSIONAL DEVELOPMENT

Caltech 5P program (in progress): 5P is a 5 component, multi-year certification to demonstrate practice in university teaching, uncluding: Pedagogy – coursework on foundational principles of undergraduate teaching, Practice – guest lecturing experience, Project – development of teaching syllabus, Professional planning – workshops on career development, Portfolio – assembling a teaching portfolio. (2023 – present, in progress).

How Learning Works: Completion of 5-session short-course focused on evidence-based principles for teaching undergraduates in STEM. Consists of lecturing and discussion components focused on academic research on effective undergraduate teaching practices. (Winter, 2024)

Transforming Your Research into Teaching: Completion of 5-session short-course focused on participants learning the skills of course design and developing a college-level course based on their area of research expertise. Topics covered included introduction to course design, designing purposeful assessment, and implementing evidence-based teaching strategies. Short-course culminated in a completed course alignment plan and a 1-slide 'chalk talk' presentation communicating key course learning goals. (Summer, 2023)

Caltech Certificate of Teaching Interest: Completion of a semester-long course and project on effective teaching, covering topics including: academic research on effective and equitable teaching, effective communication, time management during class, course design using concept mapping, among others. (2021-2022)

SKILLS

*Experimental or computational skills with significant experience *(instruments/software/methods used)*

NMR Spectroscopy: 15 years of graduate and postgraduate research experience using solution NMR Spectroscopy to study protein structure and dynamics. Including ● Isotopically labeled sample preparation (*M9 media growth*) ● Spectra collection (*Bruker Topspin*) ● Writing pulse programs (*Bruker Topspin*) ● Data analysis (*NMRpipe, NMRview, CARA, MestreNova*) ● Spectrometer maintenance (*Bruker 600, 700, 800, 900 MHz, nitrogen & helium fills, basic maintenance*). ● Isotopic labeling (*^{15}N, ^{13}C ILV, site-directed spin labeling with MTSL & MMTS, ^{19}F NMR phenylalanine & BTFA labeling*) ● Multiple dimension collection and analysis (*Diffusion ordered spectroscopy, Assignments via HNCA etc., Structure determination by NOE*) ● Paramagnetic relaxation enhancement ● Non-uniform sampling

Biophysics: ● NMR spectroscopy (*Maintaining and running Bruker spectrometers*) ● X-Ray Crystallography (*LBNL synchrotron independent use*) ● Mass Spectrometry (*Orbitrap Elite, Q-Exactive*) ● Electron Paramagnetic Resonance (*Bruker CW-EPR spectrometer*) ● Cryo Electron Microscopy (*Titan Krios*) ● Small Angle X-ray Scattering ● Fluorescence Anisotropy (*Horiba Fluorolog*) ● Bulk FRET (*Horiba Fluorolog 3*) ● Single molecule FRET (*homebuilt ALEX, Hamamatsu Photonics picosecond streak camera*) ● Fluorescence lifetime measurements (*Horiba TCSPC*) ● Mass Photometry (*Refeyn TwoMP*) ● Molecular Tweezers (*homebuilt instrument*) ● Molecular Dynamics simulations ● Circular Dichroism (*Aviv model 410*) ● Calorimetry

Molecular Biology: ● Cloning (*site-directed mutagenesis, Gibson Assembly*) ● Protein expression (*various E. coli cell lines*) ● Protein purification (*self-packed nickel/amylose/glutathione etc. columns, ion exchange and sizing columns, Chromatography with Bio-Rad Duo/NGC & GE AKTA FPLCs*) ● Immunoassays (*western blotting and quantification, immunoprecipitation*) ● Amber suppression (*Schultz method in vitro and in cell*) ● *In vitro* translation (*PURE and self-prepared systems*) ● PCR/qPCR

Biochemistry: ● Light scattering (*Beckman DU series*) ● Luciferase folding assays (*SpectraMax iD5 plate reader*) ● ATPase assays ● UV crosslinking (*Bpa*) ● Chemical crosslinking (*BMOE, BMH, DSS others*) ● Fluorescence labeling (*AlexaFluor/BODIPY maleimides*) ● EPR and NMR labeling methods (*MTSL for EPR or PRE experiments*) ● Biochemistry assays (*quantifying kinetic and thermodynamic parameters of reactions*) ● Small molecule synthesis ● Chromatography (*Agilent 1100 series HPLC*).

Software: ● NMR software (*TopSpin, NMRPipe, CARA, NMRview, MestReNova*) ● Structure visualization (*ChimeraX, Pymol*) ● CryoEM software (*CryoSPARC, Relion*) ● Protein structure determination (*PHENIX, Coot*) ● Structure computation (*Molecular Dynamics simulations in Gromacs, AlphaFold 2*) ● Bioinformatics (*Galaxy, BioPython*) ● Data analysis (*R, Matlab, Graphpad Prism*) ● Coding languages (*Python*)