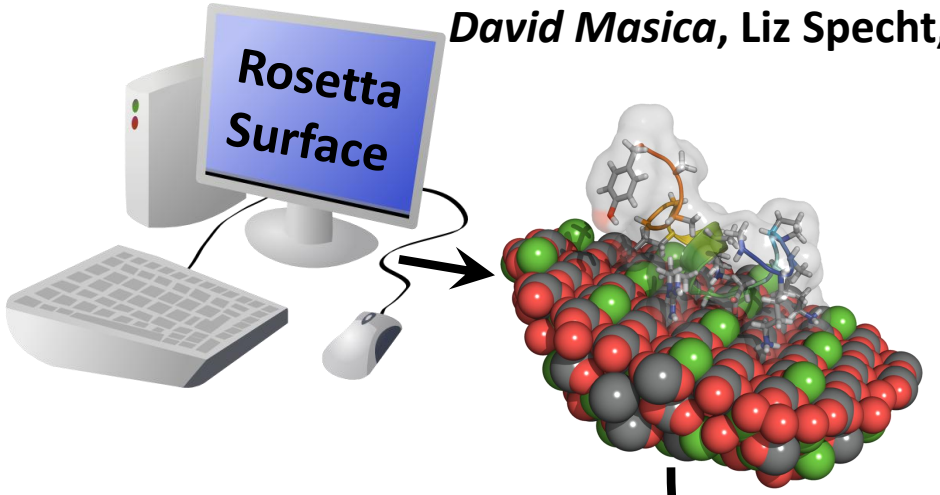
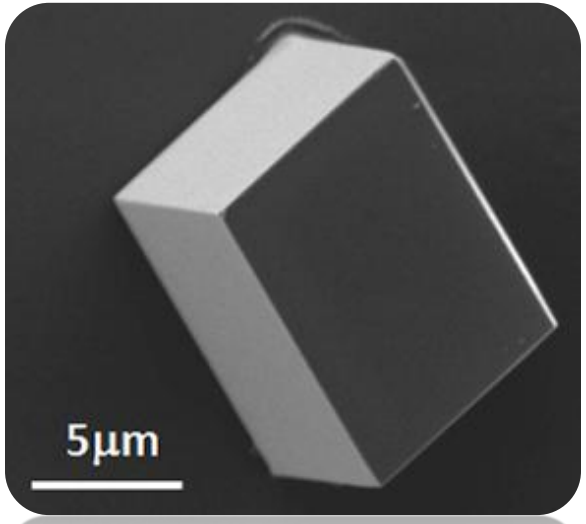
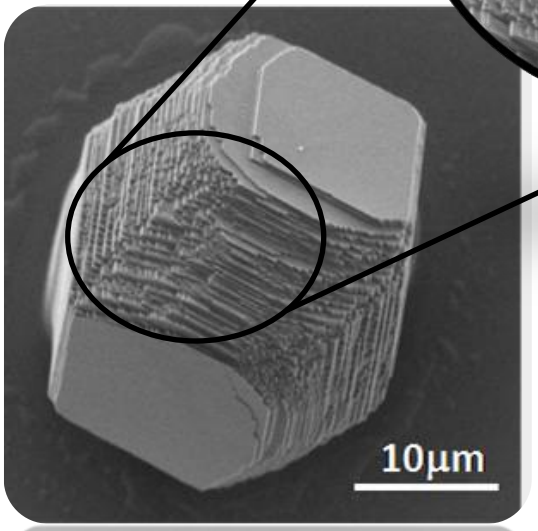
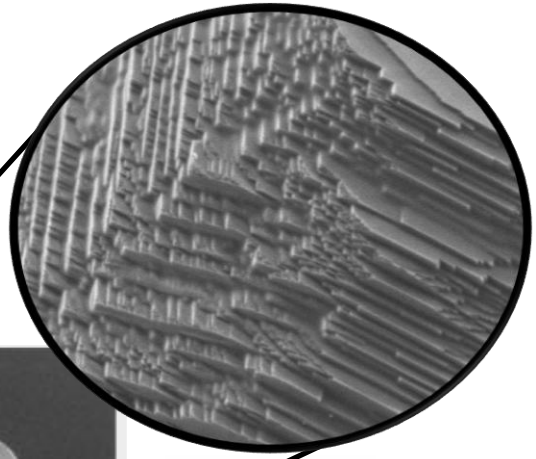
De novo design of peptide-calcite biomineralization systems

David Masica, Liz Specht, Sarah Schrier, & Jeff Gray





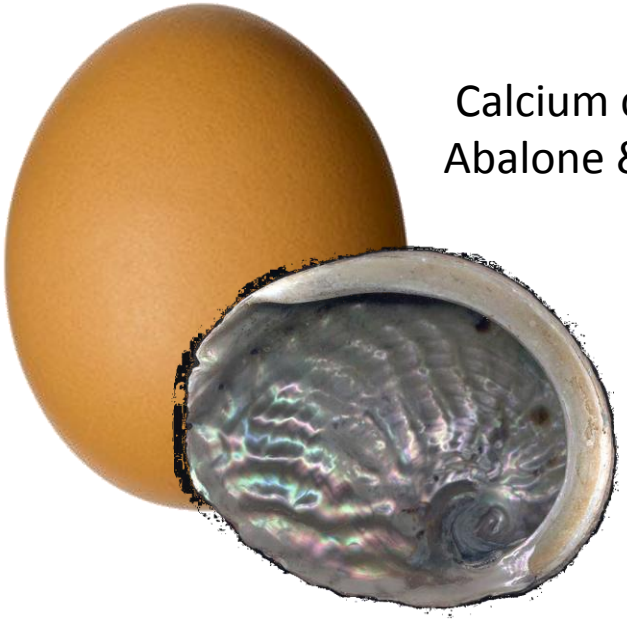




NSF

Arnold and Mabel BECKMAN FOUNDATION

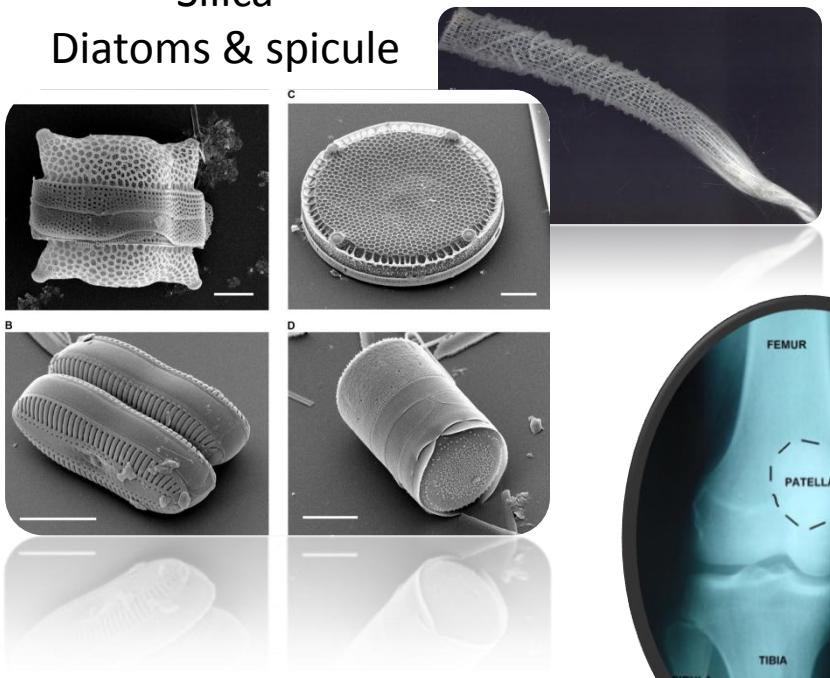
Protein biomineralization:

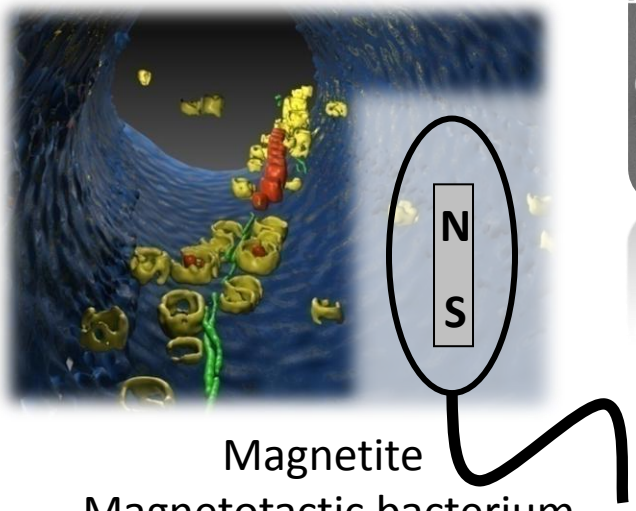


Calcium carbonate
Abalone & egg shell

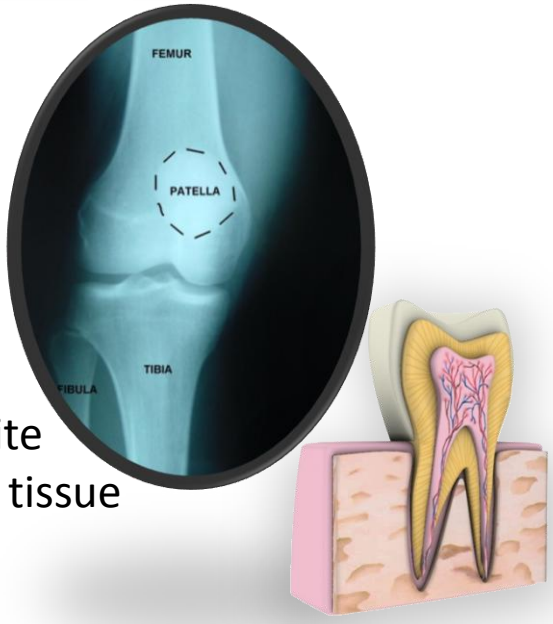
Calcium oxalate
Kidney stones

Silica
Diatoms & spicule





Magnetite
Magnetotactic bacterium

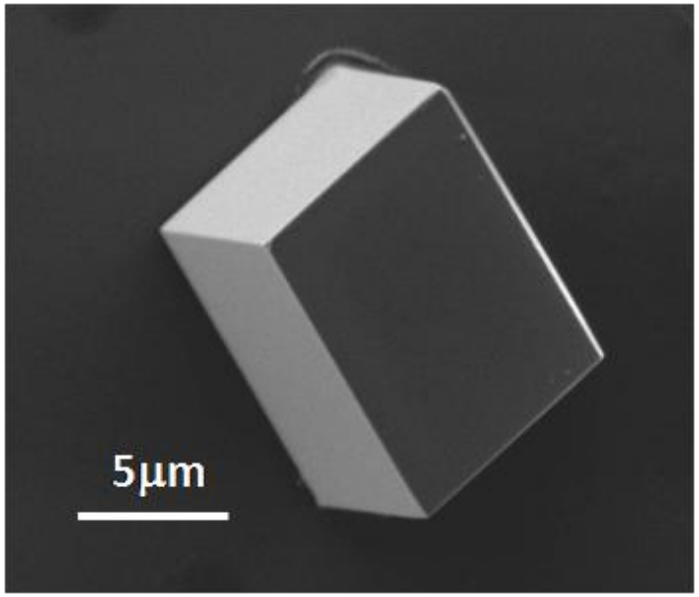


Hydroxyapatite
Skeletal & dental tissue

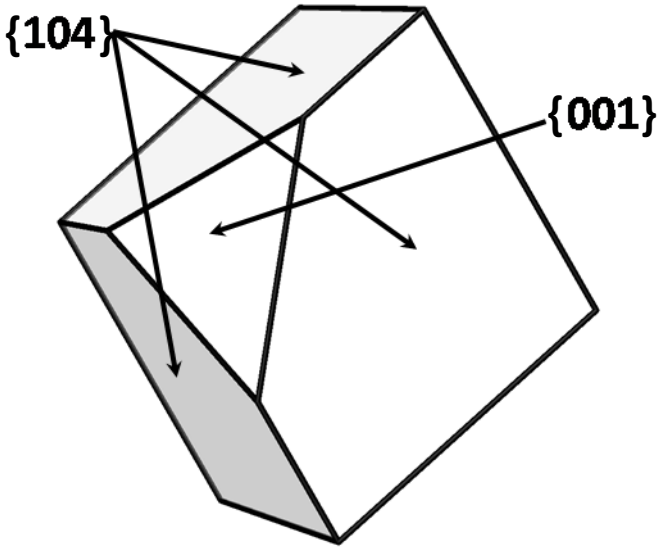
Impact and background

- Impact:
  - Utilize nature's uncanny ability to synthesize materials at physiological conditions and with nanoscale precision
  - Understand sequence determinants in hard tissue formation
- Design of biomineralization systems to date:
  - Current attempts rely on modifying naturally evolved biomineralization proteins or using directed evolution techniques to evolve material binders *in vitro*.
  - One group extrapolated sequence data from directed evolution studies to predict novel material binders
  - Currently no method of *de novo* design

Choosing the mineral and face

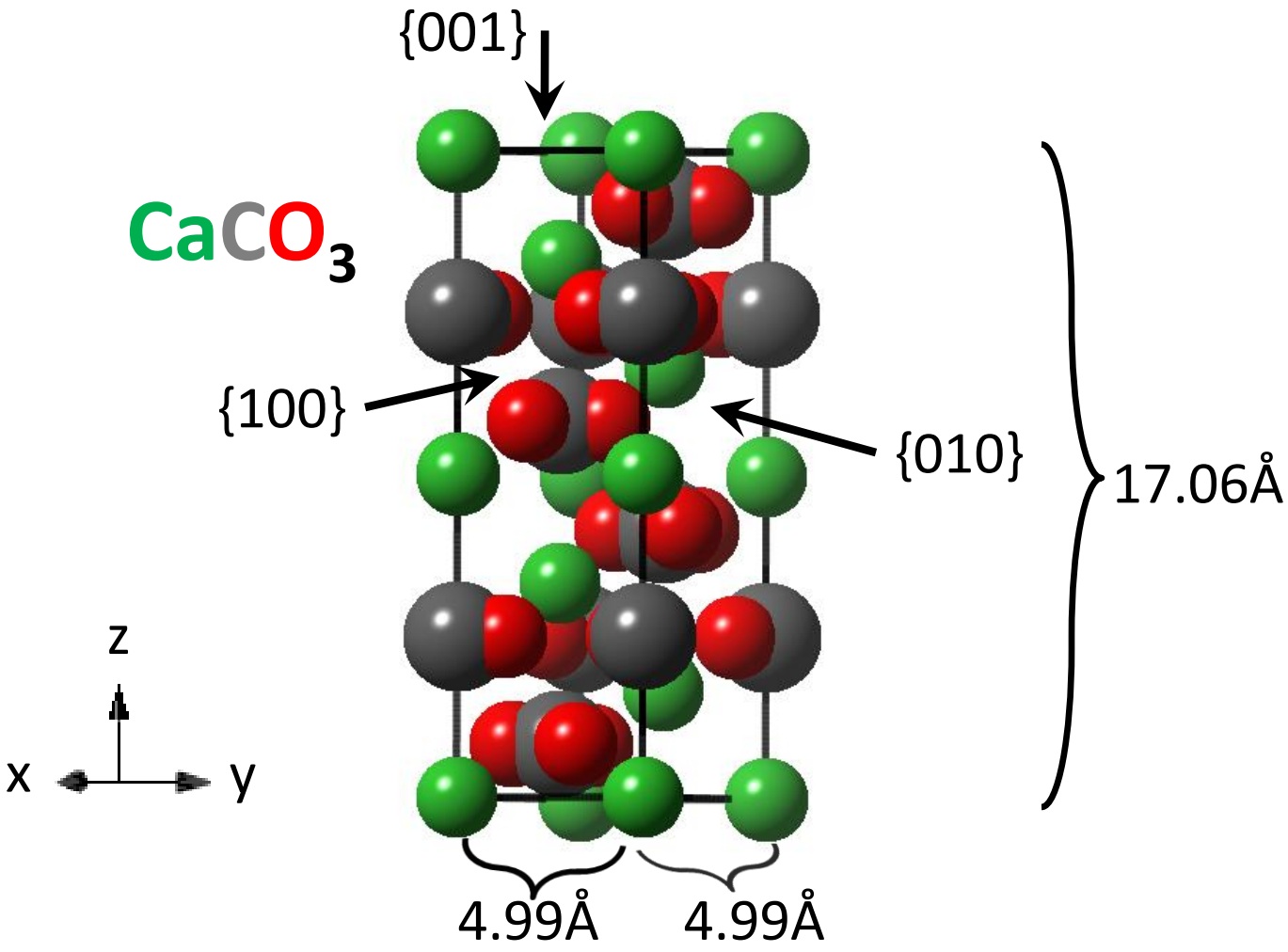


Scanning electron micrograph of native calcite crystal



Sketch of almost mature, growing calcite crystal

Choosing the surface termination

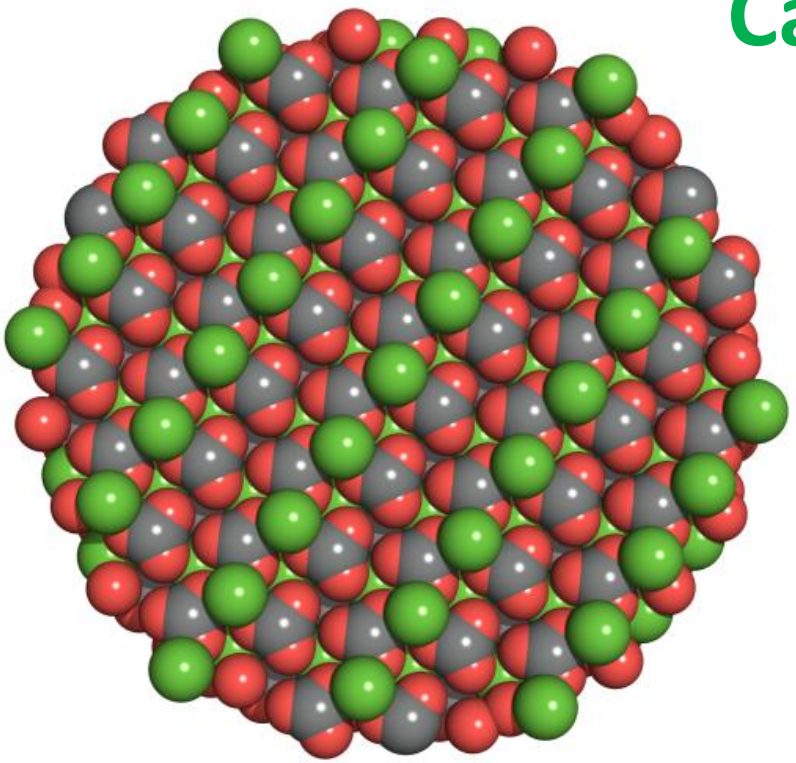


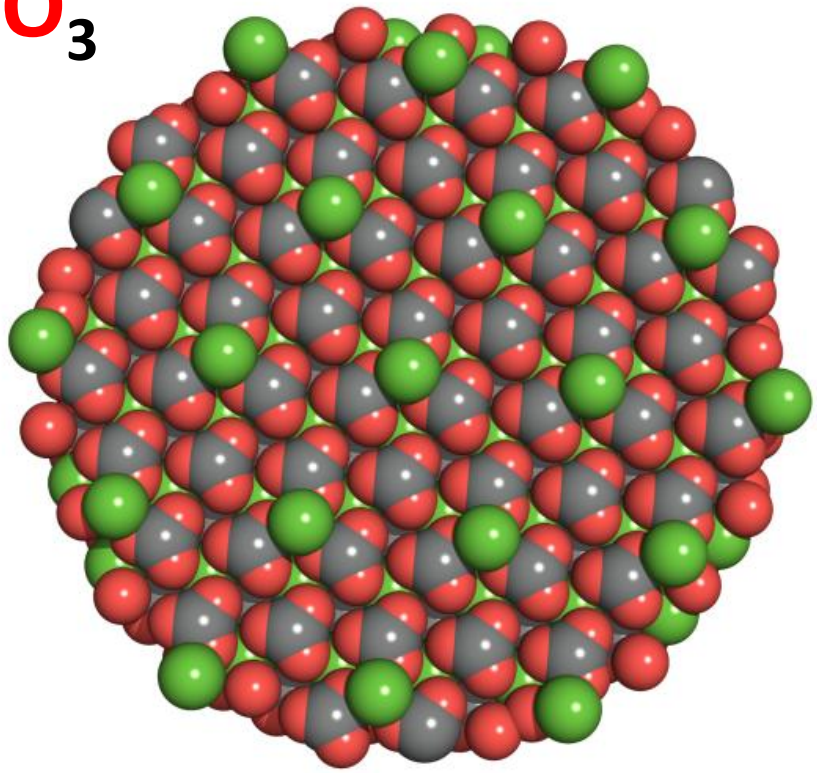
Calcite {001} surfaces with different net charge

Positive net charge

$CaCO_3$

Negative net charge



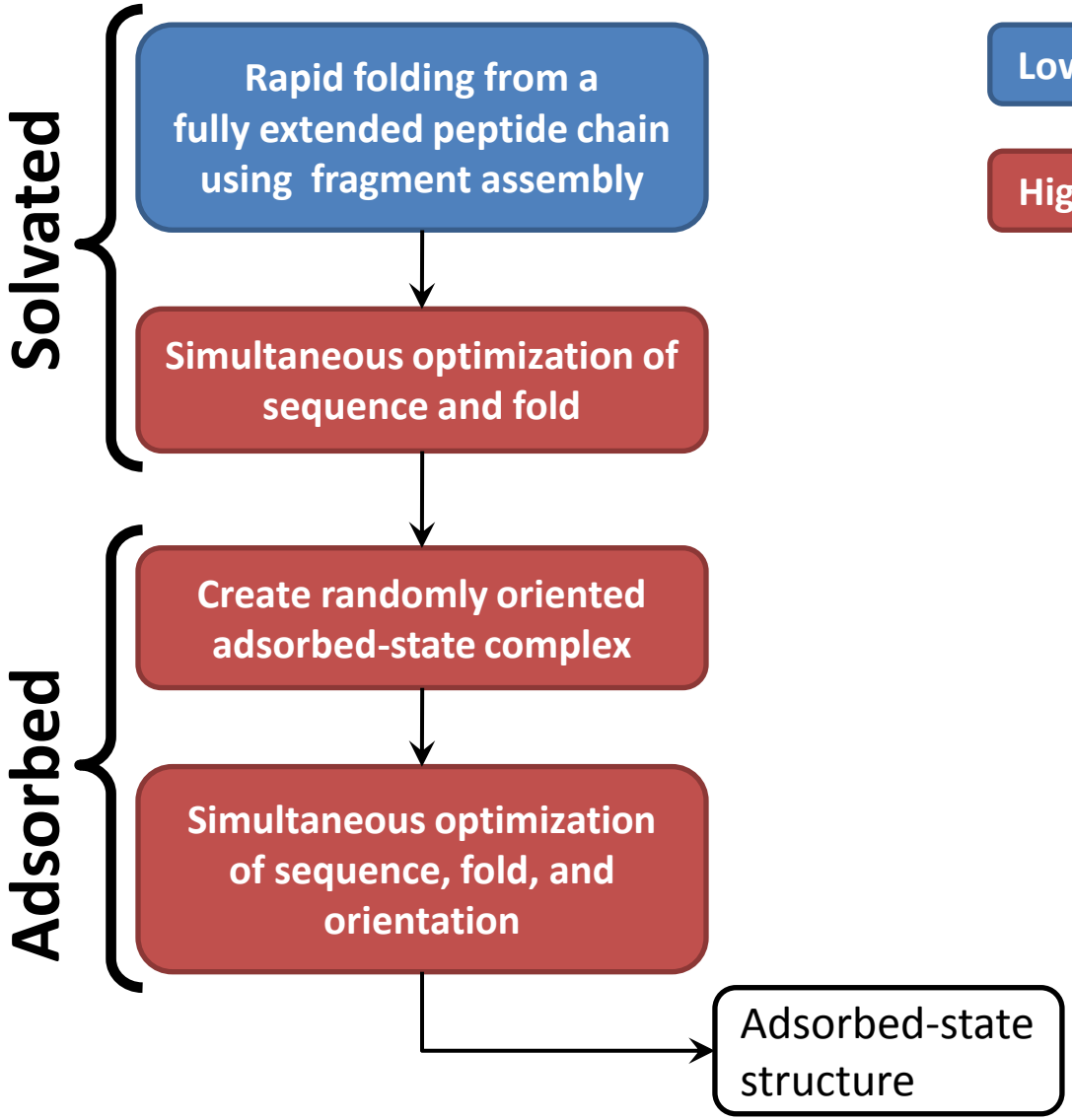


The RosettaSurface protocol

- RosettaSurface
  - Can fold a protein on a biomineral surface beginning from a fully extended peptide chain. Simultaneously optimizes side-chain, backbone, and rigid-body positions.
- RosettaSurface.NMR
  - The structure of biomineral-bound proteins cannot be determined experimentally. RosettaSurface.NMR can be combined with minimal NMR constraints to predict reasonably accurate structures of biomineral bound proteins.
- RosettaSurface.Design
  - Only method of *de novo* design in protein biomineralization

RosettaSurface.Design flow chart:

$$E_{total} = E_{att} + E_{rep} + E_{solv} + E_{hbond} + E_{electro}$$

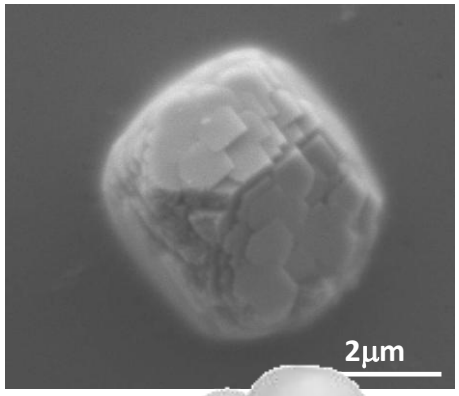


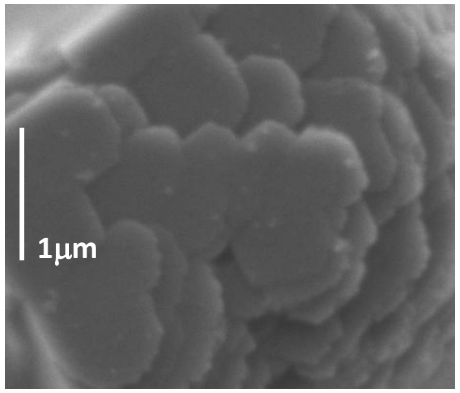
Choose sequences and synthesize

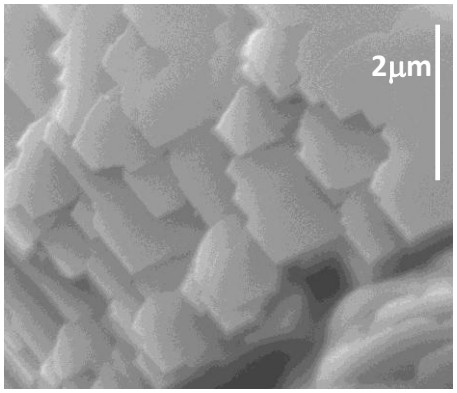
Number	Design Sequence	Scrambled Variant	Net Charge
1	GEAEGEEAAAGEGGAY	EGGAAGAEAEEGAEGY	-5
2	GEEAADAAGAEEAGAY	AAAAGAEGEGAAEEDY	-5
3	AKAPKDGRAKEGGAAY	AAGPDAKKARGGEAKY	+2
4	GAAAAARKAEKGAKAY	AARGKGAAAEKAKAAY	+3
5	APPRAKAAKAAAAGKY	AAAKAPAAGKPKARAY	+4
6	GPPPPAKAAKKAAKKY	AKAPKKAPPGAAKKPY	+5

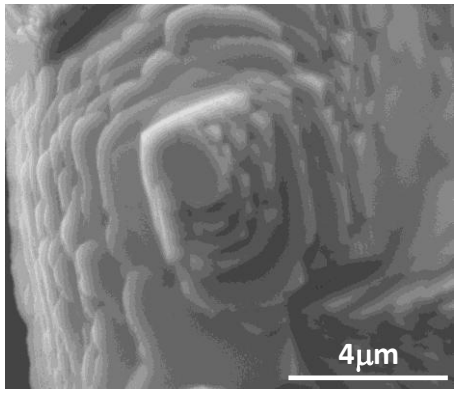
Sequence one

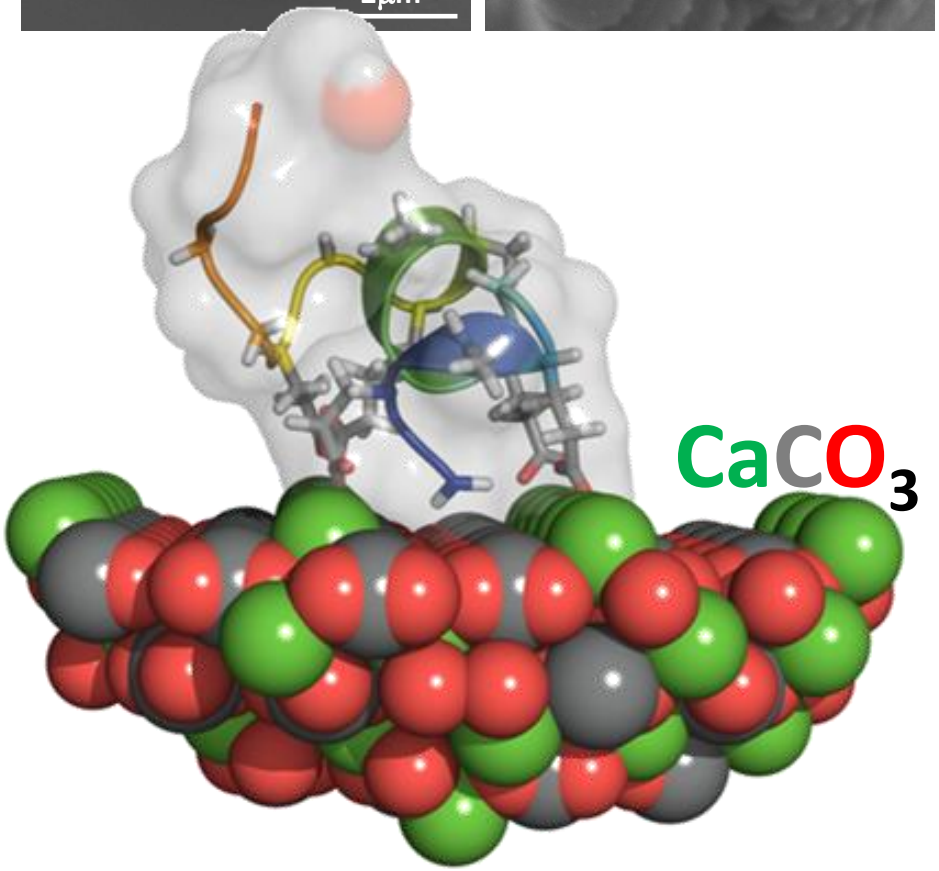
DesignOne: **GEAEGEEAAAGEGGAY** Net Charge -5 VariantOne:**EGGAAGAEAEEGAEGY**



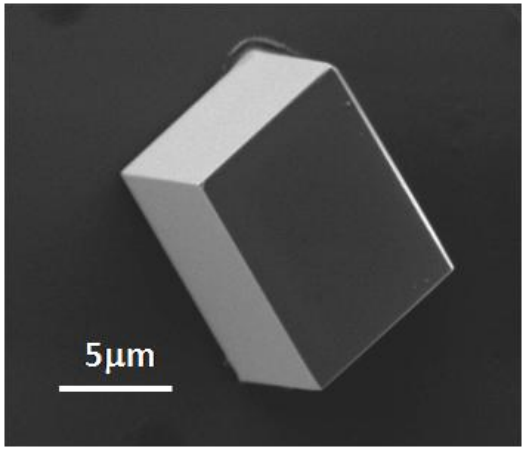






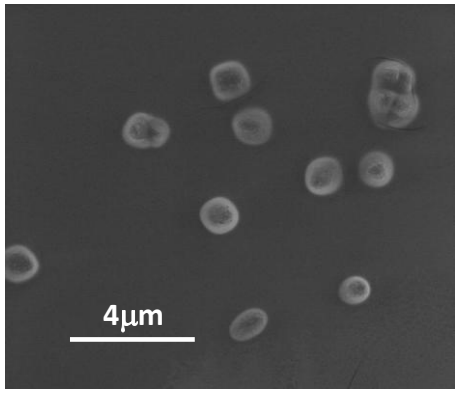


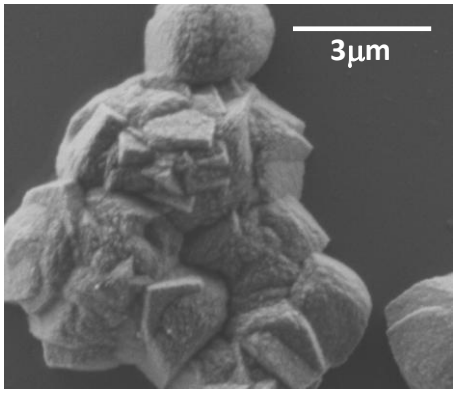
Reference crystal

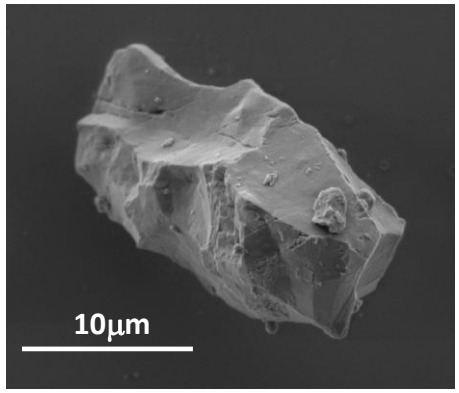


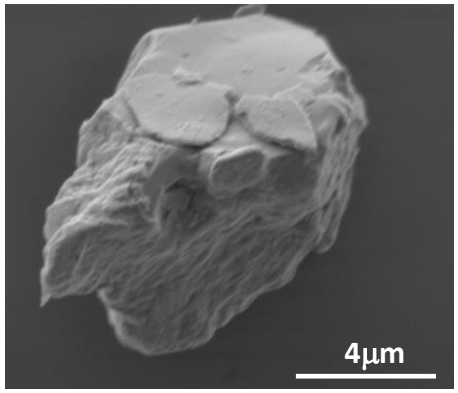
Sequence two

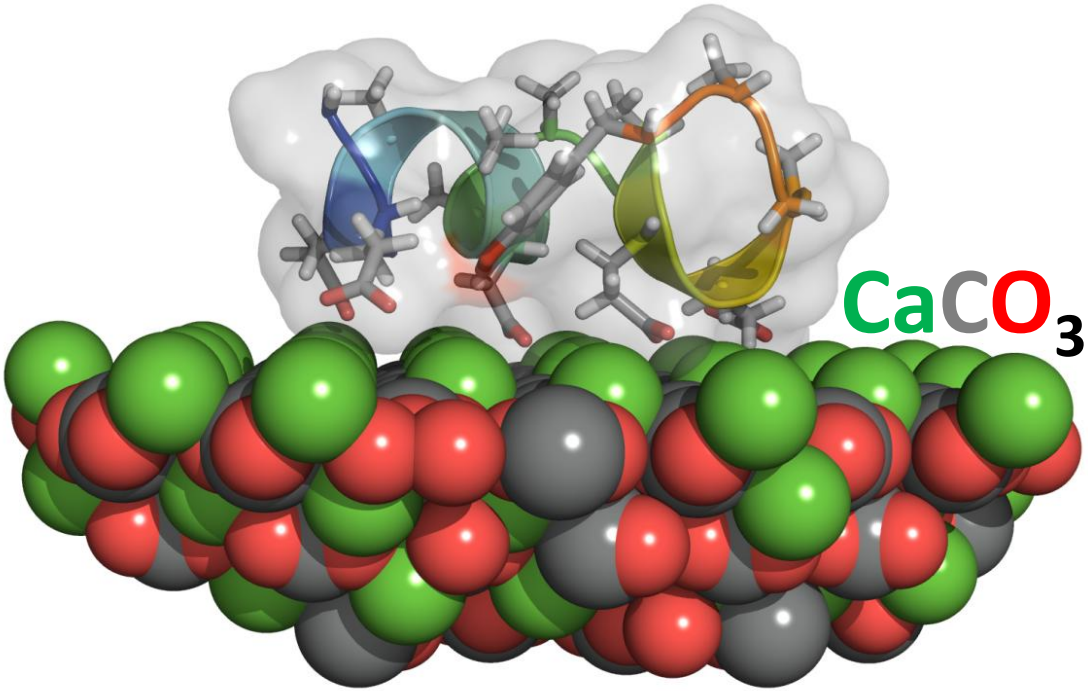
DesignTwo: **GEEAADAAGAEEAGAY** Net Charge -5 VariantTwo: **AAAAGAEGEGAAEEDY**



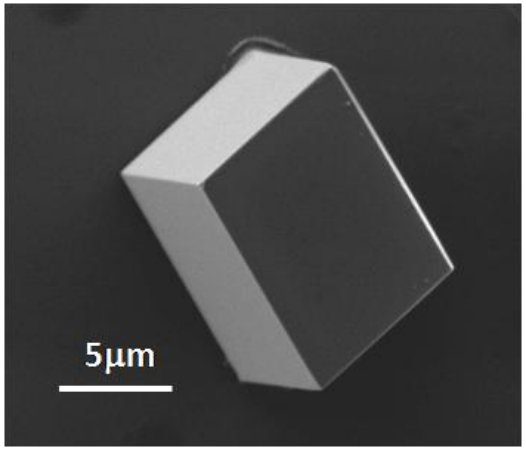






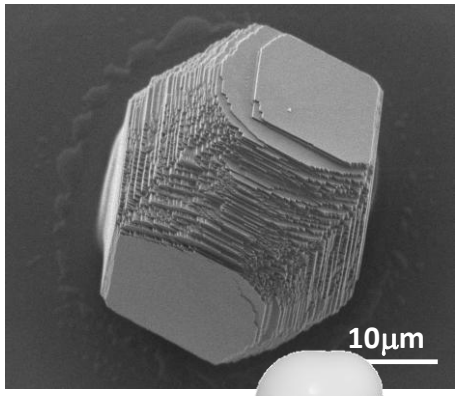


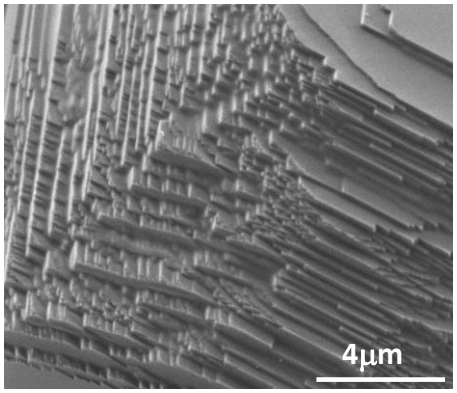
Reference crystal

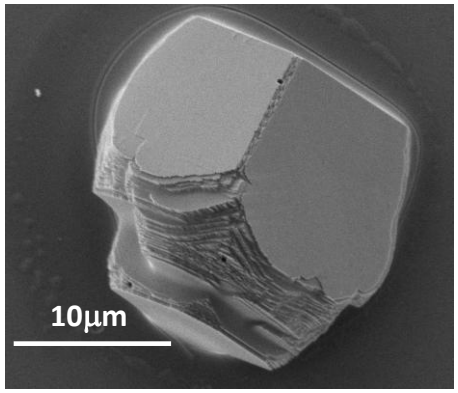


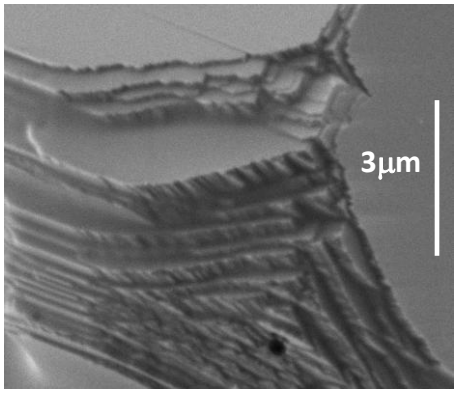
Sequence three

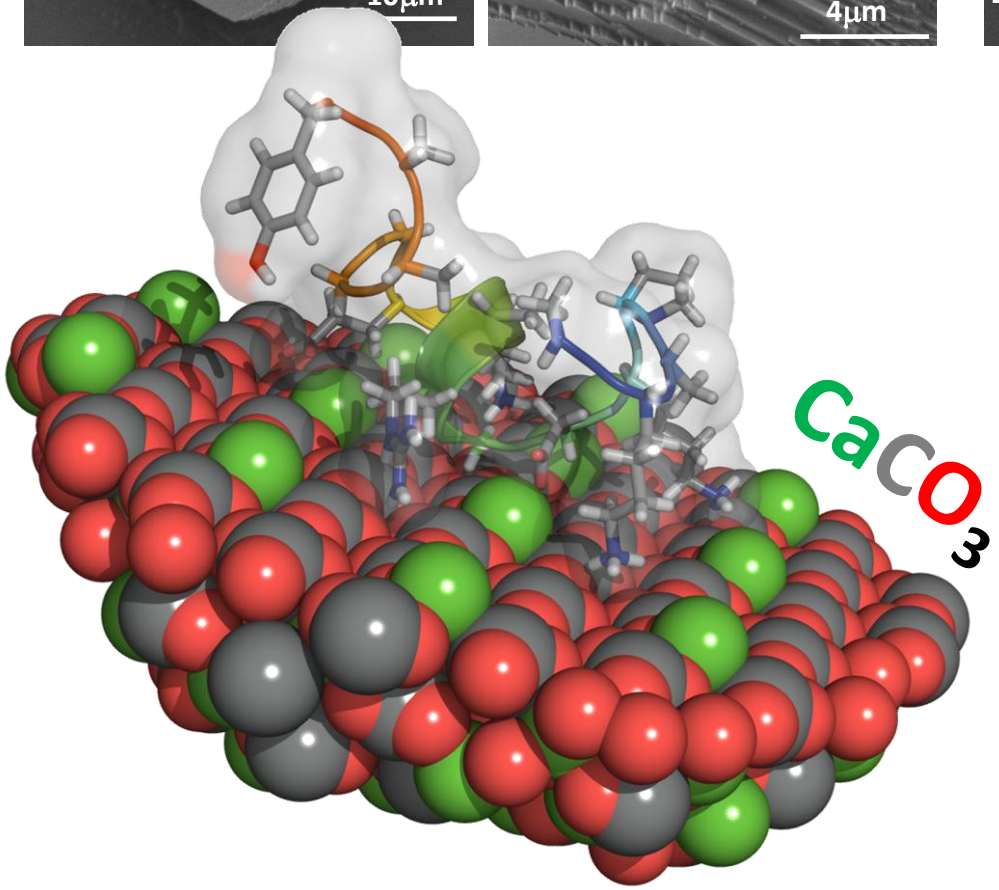
DesignThree: **AKAPKDGRAKEGGAAY** Net Charge +2 VariantThree: **AAGPDAKKARGGEAKY**



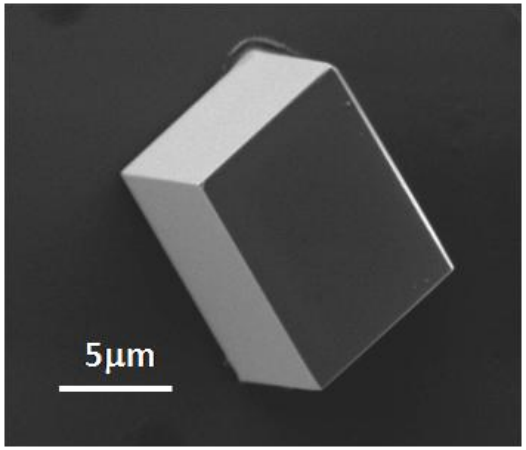






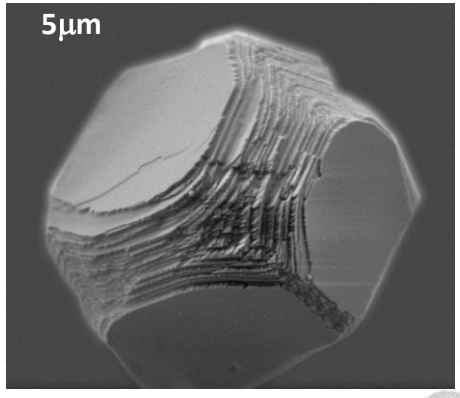


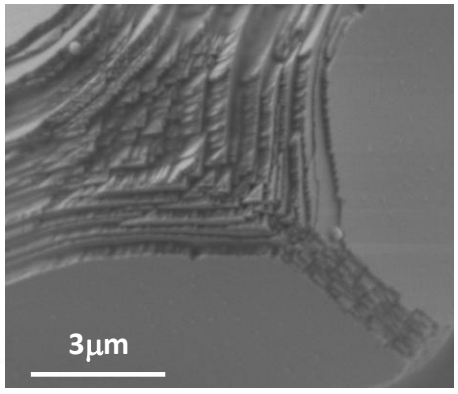
Reference crystal

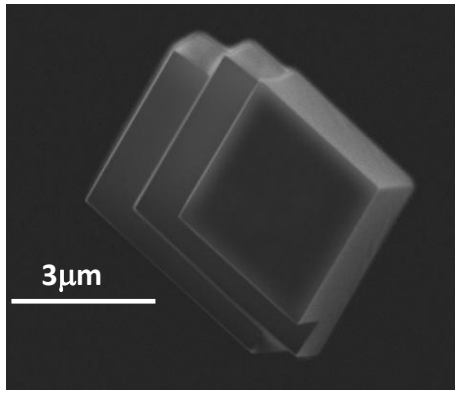


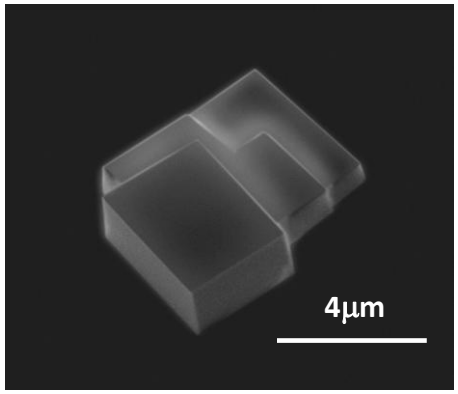
Sequence four

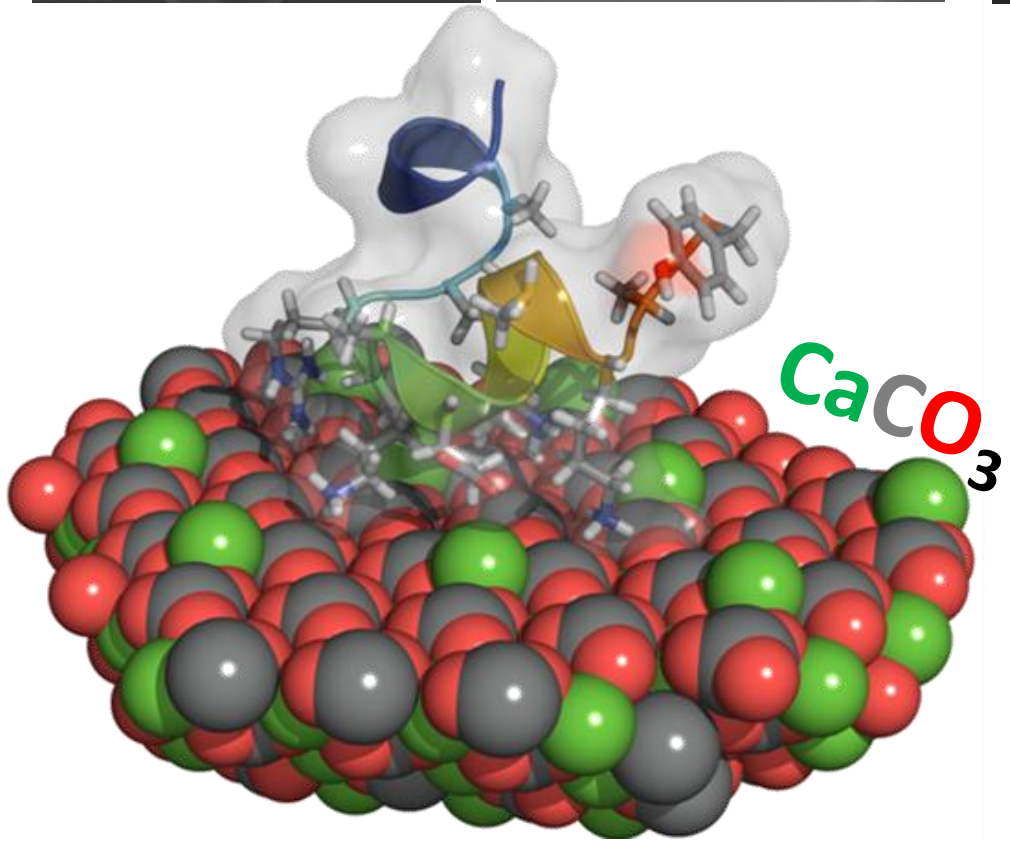
DesignFour: **GAAAAARKAEKGAKAY** Net Charge +3 VariantFour: **AARGKGAAAEKAKAAY**











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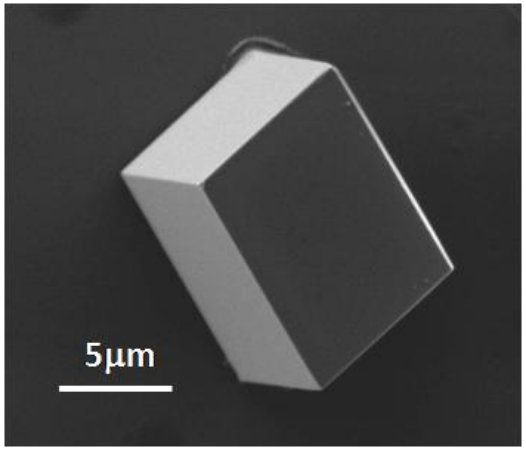
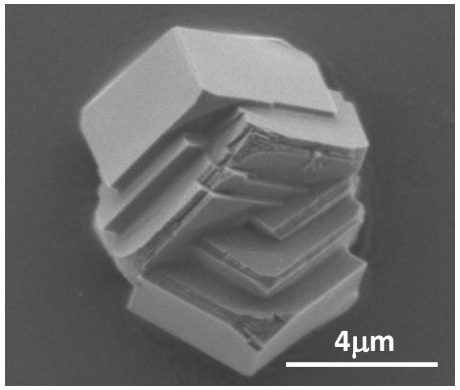
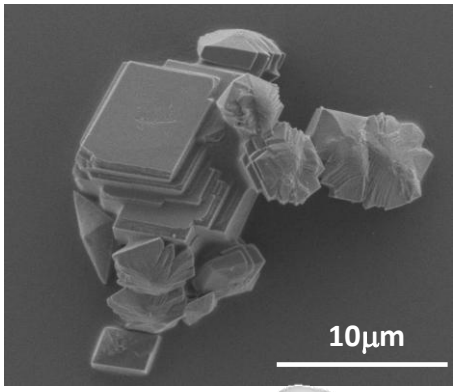
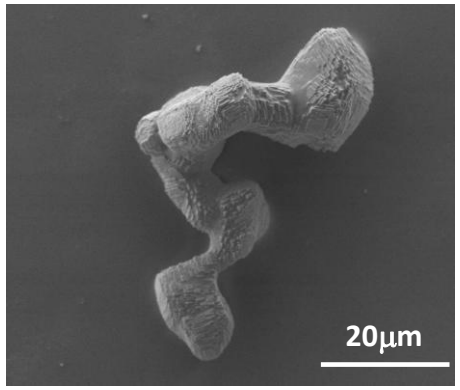


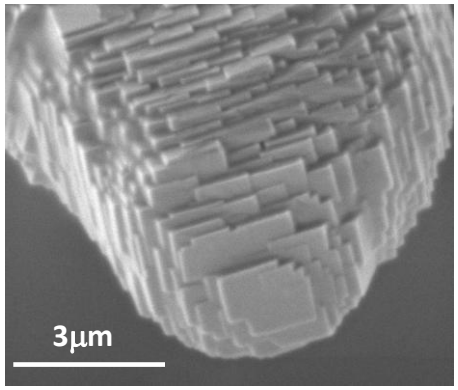
Sequence five

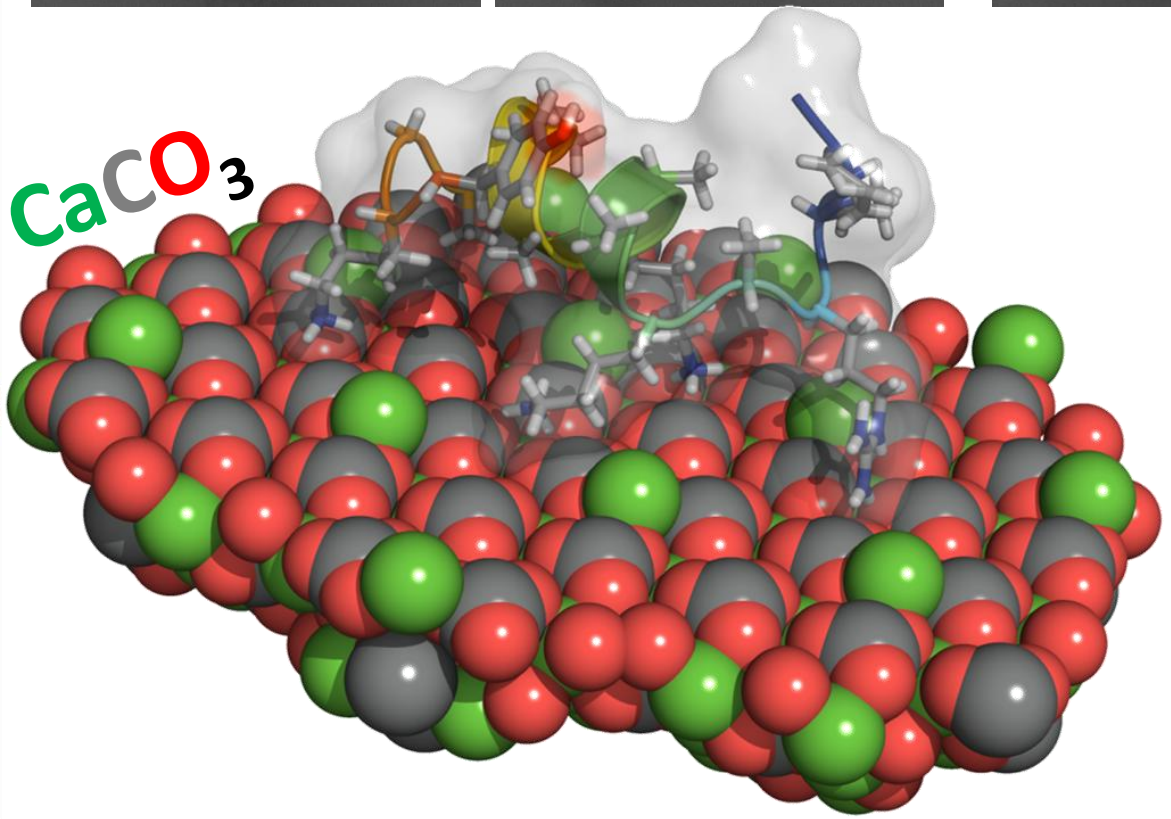
DesignFive: **APPRAKAAKAAAAGKY** Net Charge +4 VariantFive: **AAAKAPAAGKPKARAY**











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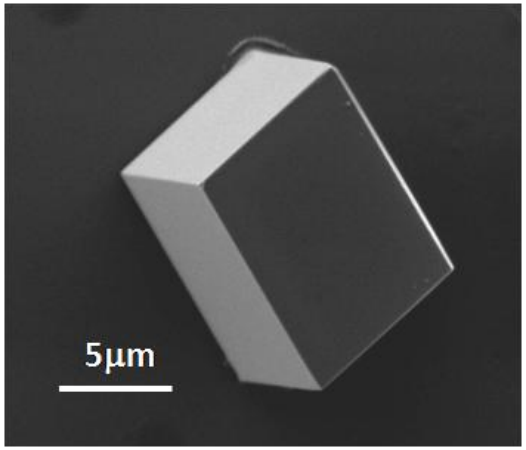
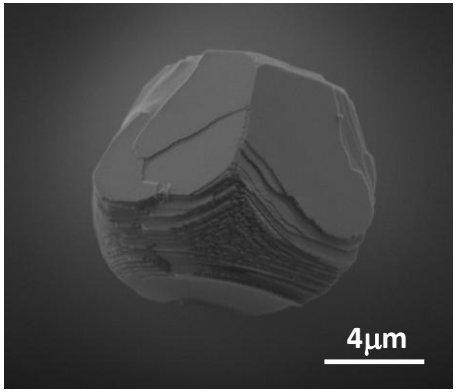


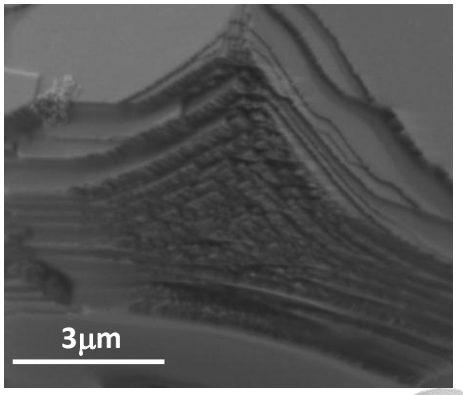
Sequence six

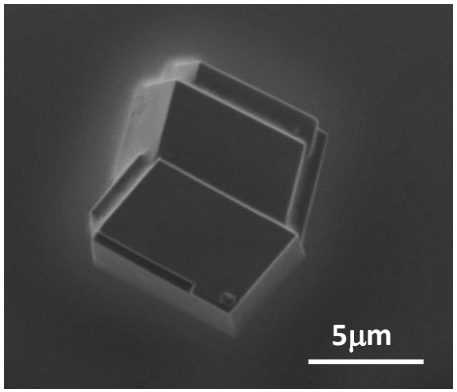
DesignSix: **GPPPPAKAAKKAAKKY**

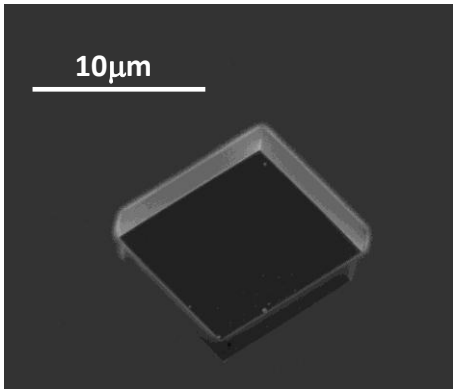
Net Charge +5

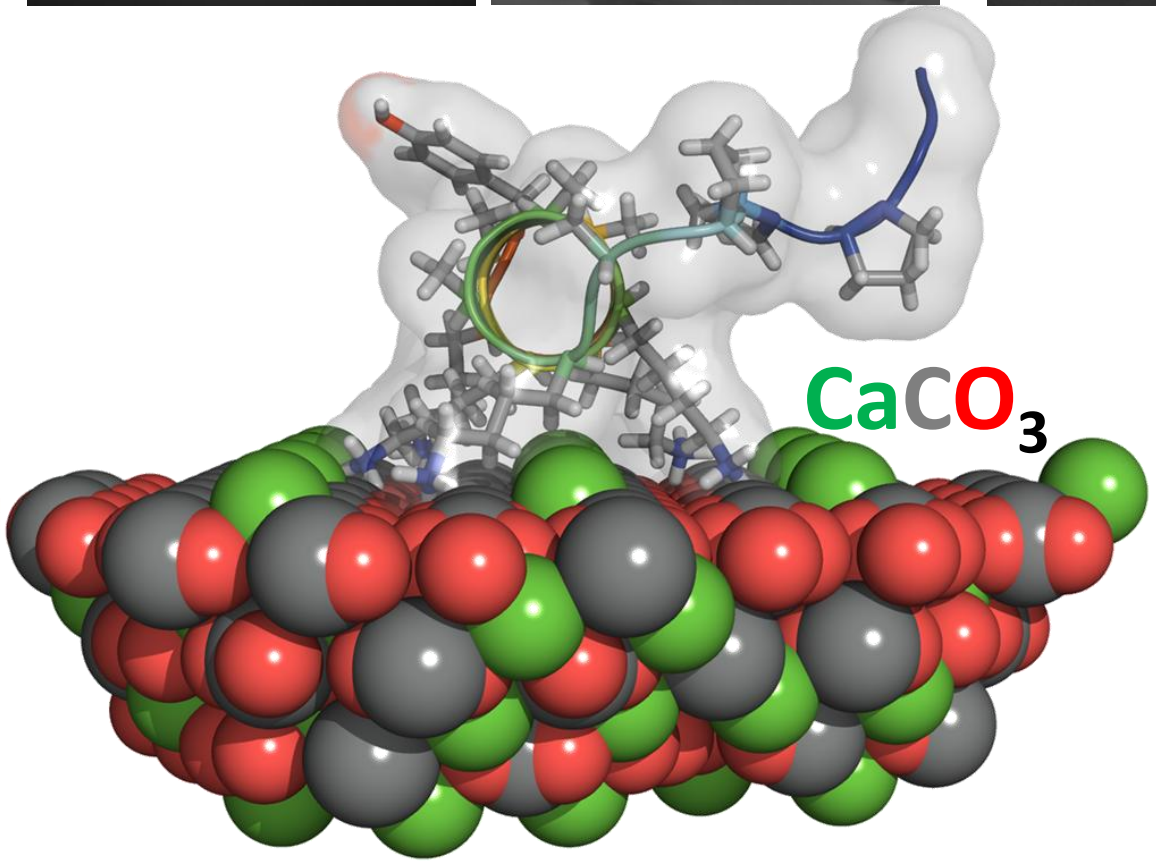
VariantSix: **AKAPKKAPPGAAKKPY**











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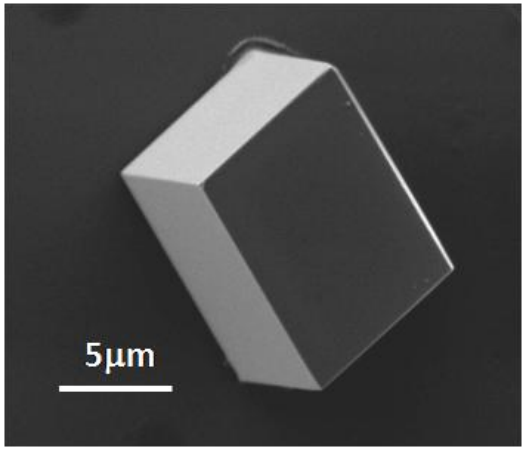

Challenges

- Many mineral-surface conformers present during mineral crystallization
  - These include transiently amorphous states
  - The structure and chemistry of these states are very similar
- Relating gross crystal morphology observed in SEM's to the atomistic data predicted by RosettaSurface.Design

Improved strategies for RosettaSurface.Design implementation?

- Consider solution state
  - Some scrambled variants were predicted, by RosettaSurface, to have lower binding energy than corresponding designs
- Design of a stable, structured protein binding domain may increase specificity.
  - Increased size
  - Add disulfide bond