Final Report on


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Executive Summary. Safe and economical disposal of 90Sr in spent reactor fuel and rapid remediation of 90Sr introduced into the environment in nuclear accidents such as the one in Fukushima in 2011 remains a technological challenge. Using funds from the ISEN booster program, we have made significant progress towards understanding sequestration of strontium in biomineralizing desmid algae. We found evidence for a “sulfate trap” mechanism in the highly unusual sequestration of strontium in desmid green algae (J. Struct. Biol. 2011). This allowed to increase the amount of strontium desmids incorporate into a (Sr,Ba)SO4 biomineral by two orders of magnitude (ChemSusChem 2011). Based on these accomplishments, we are well situated to develop desmids for removal of radioactive 90Sr from nuclear waste streams or in bioremediation of disaster sites, e.g. in Fukushima. We are currently in contact with BIOJAM, a company interested in conducting large-scale trials of the technology we developed.

Publications from this project had exceptional visibility through an editorial in ChemSusChem, coverage by Nature News of a talk given by graduate student Minna Krejci at the ACS meeting, and articles on Forbes.com, Phys.org, and others. Minna’s second publication in the Journal of Structural Biology was selected as a cover article and her talk at the 11th International Symposium on Biomineralization 2010 was awarded the best student talk award. Her work was highlighted on the APS website.
1. **Personnel:**
   1.1. Minna Krejci (Ph.D. 2012)
   1.2. Brian Wasserman (undergraduate research assistant)
   1.3. Pongkarn Chakthranont (undergraduate research assistant)

2. **Collaborators:**
   2.2. Lydia Finney, Advanced Photon Source, Argonne National Laboratory.
   2.3. Ian McNulty, Advanced Photon Source, Argonne National Laboratory.
   2.4. D. Legnini, Advanced Photon Source, Argonne National Laboratory.

3. **Publications:**

4. **Presentations (2 invited talks, 3 contributed talks, 6 posters)**
5. **Follow-up Proposals:**

5.1. Alumna of Northwestern University 2009 (granted)

5.2. NSF CAREER 2009 (declined)

5.3. ISEN-Argonne 2010 (declined)

5.4. Northwestern-Argonne Early Career Investigator Award 2011 (declined)

5.5. Extensive discussions with DoE program officers revealed that DoE remediation programs focus on subsurface organisms, ruling out use of algae. Interest in algae at DoE is limited to biofuels production.

**Introduction.** Nuclear power can drastically reduce carbon dioxide emissions and reduce our dependence on fossil fuels. However, its sustainability depends on the ability to safely reprocess spent fuels and dispose of high-level nuclear waste such as $^{90}$Sr. Vitrification in a glass sarcophagus and safe underground storage depends on the ability to separate $^{90}$Sr from low-level waste. Current research focuses on inorganic ion-exchange materials. Despite great progress, however, the search for a truly selective and cost-efficient process continues. In view of the existing volume of nuclear waste, waste generated by de-commissioning nuclear power plants or nuclear weapons, and with the added threat of accidental release from nuclear power plants as a consequence of natural disasters, in acts of war, or by terrorism, there is a pressing need for innovation in the field of selective binding and separation.

In our bodies, the chemical similarity of Ca$^{2+}$, Sr$^{2+}$, and Ba$^{2+}$ leads to indiscriminate transport by almost all known transport proteins. Consequently, $^{90}$Sr is incorporated into bone, and increased cancer mortality. Surprisingly, there are a few organisms that are able to selectively sequester Sr and Ba in biominerals (Figure 1). For example, the radiolarian Acantharea build endoskeletons from single crystalline celestite (SrSO$_4$). Crystalline barite (BaSO$_4$) is deposited for example in vacuoles by the desmid green algae, and the ciliate genus *Loxodes*. The accumulation of Sr and Ba in the presence of up to 5 orders of magnitude excess Ca emphasizes the great selectivity frequently observed in biological ion trafficking, unmatched even by the most advanced ion exchange materials. Clearly, there is much to be learned from the sequestration strategies these organisms have evolved. Despite this, the mechanisms by which Sr or Ba are sequestered in these organisms remains underexplored. This is in part due to the very challenging problem of quantifying and visualizing the chemically very similar ions Ca$^{2+}$, Sr$^{2+}$, and Ba$^{2+}$. Ratiometric imaging using ion-selective fluorescent probes is widely used to localize and quantify the concentration of Ca$^{2+}$ and other metal ions. However, there are no fluorescent dyes selective for either Sr$^{2+}$ or Ba$^{2+}$ in the presence of physiological levels of Ca$^{2+}$. Synchrotron X-ray fluorescence microscopy (XFM) at third-generation sources such as the APS, on the other hand, combines extremely low minimum detection limits ($>10^{-20}$ mol/µm$^2$) with subcellular resolution (>150 nm), without the need for exogenous probes. Unlike optical microscopy, it can be used to quantify many elements at the same time.

**Figure 1:** A: The endoskeletons of the marine, unicellular *Acantharea* are composed of single crystals of SrSO$_4$. B: The ciliate *Loxodes magnus* uses spherulites of barite (BaSO$_4$) (arrowhead) in its gravity-sensing Müller bodies (arrow). C: In the desmid green algae of genus *Closterium*, vacuoles at the tip (arrow) contain barite crystals.
We have made significant contributions to the understanding of biological sequestration of strontium and barium in biomineralizing desmids, a ubiquitous freshwater green alga. Using a combination of X-ray fluorescence microscopy (XFM) and analytical chemistry, we have shown that desmids use a sulfate trap mechanism to co-precipitate strontium and barium in the form of a solid solution (Sr,Ba)SO$_4$ with barite structure. Based on our understanding of the biological mechanism and the Lippman phase diagram of this solid solution we were then able to design culture conditions for desmids under which strontium sequestration is increased by several orders of magnitude. Further investigation of Sr transport by XFM tomography in desmids revealed the importance of vesicles of the endomembrane system (Figure 2).

![Figure 2](image.png)

**Figure 2.** Identification of organelles in SXRF maps. (A) High Zn concentrations in the nucleus of the cell (arrow), and pyrenoids are visible in the S map (arrowhead). The lobes of the chloroplast can be seen in the Fe map, which appear to be interdigitated with ridges of high Sr concentration. A line plot (from a to b in Sr/Fe maps) hints that Sr concentrations are highest in the grooves between the chloroplast lobes (cf. Figure 1A, B). (B,C) tomography confirms this analysis and allows to estimate volume concentrations. Drying artifacts, however, may have caused rearrangement of ions and may affect local concentrations.

During our XFM investigation, we serendipitously uncovered a very interesting pattern of Mn compartmentalization at the growth tips of *C. moniliferum* (Figure 3). As a nutrient integral to photosynthesis, Mn plays a particularly important role for algae and higher plants. Manganese deficiency is an agricultural concern. On the other hand, manganese can be growth-limiting or toxic if present at excessive concentrations. Using XFM and ICP-AES, we determined uptake and localization of Mn in the desmid green algae *C. moniliferum* and found that the organism is a hyperaccumulator. Using Mn K-edge µ-XANES analyses on frozen-hydrated cells, we identified the cell tip deposits as primarily Mn(II). We further found evidence that the Mn deposits are related to the high pectin content of the cell wall in the cell tip region. Hyperaccumulation of Mn indicates that *Closterium* may be able to tolerate high concentrations of other heavy metals. This is an advantage for remediation of nuclear waste and an interesting target for future study. Furthermore, *C. moniliferum* is well-known for its striking induced radiation resistance, where cells are more resistant to radiation if they are previously exposed to an initial low dose. This could be a consequence of the high ratio of Mn to Fe (Mn/Fe = 0.25) that rivals that of the highly radiation resistant bacterium *Deinococcus radiodurans* (Mn/Fe = 0.24).

![Figure 3](image.png)

**Figure 3.** XFM elemental maps of *C. moniliferum*. A) Ca, Mn, and Fe maps of a whole cell. Mn is highly localized to the cell tips (arrows). Fe also appears to accumulate in these areas, to a lesser extent. Ca concentrations also appear slightly elevated in the tips. All concentrations are given in fmol/µm². Imaging was performed at 10 keV with 0.5 µm step size and 1 s dwell time.